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https://doi.org/10.1016/j.toxlet.2024.07.540

P13-25
Evaluating the effects of food additive E171 on colorectal cancer risk: a human dietary intervention study

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Background: The widespread use of the food additive E171 (titanium dioxide, TiO_2) as a white colorant, particularly in products like sweets and chewing gum, raises public health concerns due to its potential link to colorectal cancer (CRC) and reduced colorectal health. E171 has been shown to induce genotoxic effects *in vitro* and enhance tumor formation in animal models for colitis. Other findings suggest that E171 might be involved in the induction of inflammation in the colon.

Objective: This human dietary intervention study primarily investigates the impact of E171 on whole genome gene expression profiles in rectal biopsies from healthy volunteers, fecal microbiome changes, and effects on systemic and local oxidative stress and inflammatory markers from plasma, blood, and biopsies. Our study aims to bridge the gap between the results obtained by *in vitro* and animal models used for human health risk assessment and the human situation.

Study Design: We employed a randomized crossover design; this four-week study included healthy subjects randomly assigned to intervention groups. The study protocol entailed a daily regimen wherein participants consumed yogurt containing E171 at a relevant human oral intake of 2.0 mg/kg_{bw}/day for 14 days. Subsequently, biopsies, blood, and fecal samples were gathered for analysis. In the study's control phase, participants consumed yogurt without E171. Adherence to the diet was monitored and confirmed via daily dietary assessments.

Study Population: We recruited 31 healthy volunteers who completed the intervention between May 2022 and February 2024 in the Limburg Region, The Netherlands. The participants were, on average, 31.2 (±14.2) years old and had a Body Mass Index (BMI) of 23.1 (±2.0).

Results: We determined the effect of oral intake of E171 on systemic markers for oxidative stress (TEAC, PCCs, TBARS, and superoxide levels) and inflammation (hs-CRP, SAAs, ILs). Whole genome gene expression analysis using Next Generation Sequencing revealed differentially expressed genes, comparing paired measurements before and after the intervention in samples from the same individuals. These DEGs were used for pathway analysis and constructing a gene interaction network, demonstrating the key molecular processes involved in the response. The comparison between these findings in humans and the previous *in vitro* and animal data shows the relevance of these models in evaluating potential risks associated with dietary intake of E171.

Conclusion: This study presents the first *in vivo* results of the effects of E171 on humans through comprehensive genomic, oxidative stress, and inflammatory marker analysis. Our findings illuminate the interactions of dietary E171 exposure on human colon health and its potential role in CRC risk. These insights are pivotal for informing public health policies and guiding regulatory actions concerning E171.

https://doi.org/10.1016/j.toxlet.2024.07.541

P13-26
Unveiling the therapeutic potential of cannabinoids in ER$^+$ breast cancer: cytotoxicity and endocrine activity profile

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Introduction: The therapeutic potential of cannabinoids (CBs) has been explored for various diseases and clinical conditions, including cancer [1]. Regarding breast cancer (BC), research has been mainly focused on triple negative and HER2$^+$ tumors [2]. Recently, our group showed that Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) present important anti-tumor effects in estrogen receptor-positive (ER$^+$) BC [3], the most diagnosed BC subtype. Moreover, we verified that CBD could improve exemestane efficacy in BC cells, which may represent a major progress in ER$^+$ BC treatment and in the management of endocrine resistance [4]. However, THC and CBD are just two of more than 120 phytocannabinoids found in the *Cannabis* plant, for which therapeutic properties are yet to be explored. Here, we evaluated the cytotoxic effects and the behavior as ER and androgen receptor (AR) modulators of 10 CBs in non-cancer and cancer models.

Methods: The CBs studied were cannabidiol (CBD), cannabigerol (CBG), cannabidivarin (CBDV), cannabinol (CBN), cannabidiol-C4 (CBDB), cannabidiolic acid (CBDA), cannabidiol monomethyl ether (CBDM), cannabichromenic acid (CBCA), cannabigerovarinic acid (CBGVA) and cannabichromene (CBC). Cytotoxic properties were assessed through MTT/LDH assays in non-tumor cells, human normal mammary epithelial cells (MCF-10A) and human foreskin fibroblasts (HFF-1), and in human ER$^+$ BC cells (MCF-7aro). The binding of CBs to aromatase, ER and AR was initially assessed *in silico*, followed by transactivation assays used to profile their effects on human ER and AR. Moreover, aromatase inhibition was assessed in a radiometric assay using human placental microsomes.

Results: Results demonstrated that all CBs studied, except CBG and CBDM, decreased MCF-aro cell viability, with LDH release detected for CBDB and CBGVA. Moreover, CBD, CBG, CBDV, CBN and CBC are safe for non-cancer cells. Regarding endocrine modulation, most of the *in silico* predictions were confirmed *in vitro*. CBD was the only CB able to inhibit aromatase though all CBs acted as AR antagonists with inverse agonist properties. Additionally, CBDV, CBDB and CBDM displayed agonist activity in ER, while CBDA, CBCA and CBC acted as inverse agonists. CBD and CBN acted as ER antagonists with inverse agonist properties.

Conclusions: Despite the increasing number of studies regarding the benefits of CBs for the treatment of different cancers, information on ER$^+$ BC is still scarce. Here we showed that despite the harmful effects of some CBs in non-tumor cells, they are able to disrupt endocrine signaling, which might be valuable for improving treatment of ER$^+$ BC cases. Thus, this work presents, for the first time, a comprehensive

study regarding CBs and ER+ BC, reinforcing the anti-tumor actions attributed to them and paving the way for novel therapeutic approaches.

Acknowledgments: FCT: UI/BD/151314/2021; DL 57/2016 – Norma Transitória: SFRH/BPD/98304/2013; UIDP/04378/2020; UIDB/04378/2020; LA/P/0140/2020

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https://doi.org/10.1016/j.toxlet.2024.07.542

P13-27
Toxicological evaluation of verbascoside from the viewpoint of genotoxicity

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Verbascoside is phenylethanoid glycoside type plant phenolic compound which is predominantly found in some medicinal plants belonging to Verbenaceae, Oleaceae, Buddlejaceae, Lamiaceae, and Scrophulariaceae families [1]. Research has established that verbascoside exhibits a wide array of biological activities, including anti-inflammatory [2–3], anti-ulcerogenic [4], antispasmodic [5], antioxidant [6], antimicrobial [7], and analgesic effects [8]. While many studies have investigated these activities, not enough research has been done on its effects on DNA and whether it can cause or prevent mutations. This study aims to address this gap by investigating the mutagenic/antimutagenic and genotoxic/genoprotective potential of verbascoside.

Verbascoside used in this study was previously purified from *Globularia sintenisii*, and its structure elucidation was performed by NMR[9]. The Ames Assay, using the standard plate incorporation method, was performed to evaluate its mutagenicity/antimutagenicity following OECD test guideline 471. *Salmonella typhimurium* tester strains TA98 and TA100 were used to determine frame shift and base pair mutations, with and without a metabolic activation system. Micronucleus and comet assays were performed in the CHO cell line to evaluate the genotoxic/genoprotective potential of verbascoside.

Results revealed that verbascoside does not induce mutagenicity in both TA98 and TA100 strains with or without metabolic activation. Also, it did not cause genotoxicity in CHO cells in both micronucleus and comet analyses.

On the other hand, no significant decrease was observed against direct and indirect mutagens, indicating the lack of antimutagenic activity in the tested strains. Co-treatment of doxorubicine as a well-known genotoxic compound with verbascoside led to a decrease of the doxorubicine-induced micronuclei, which ranged between 21 and 37% in both micronucleus and comet assays. However these effect were not found dose dependent and statistically significant.

The findings of the present study provide scientific basis to the safety of verbascoside from the viewpoint of genotoxicity risk, and in fact, it was found to be beneficial against genotoxicity.

Acknowledgement: This study was financially supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK BİDEB 2209A, Project No:1919B012217189).

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https://doi.org/10.1016/j.toxlet.2024.07.543

P13-28
Lifetime carcinogenicity studies in Han Wistar and Sprague-Dawley rats: historical data for survival, neoplasms and causes of premature death

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Available survival and tumor incidence historical data are useful for interpreting lifetime carcinogenicity bioassays. These data were compiled for control Sprague-Dawley (SD) and Han Wistar (HW) rats in eight 104-week carcinogenicity studies between 2006 and 2018 at CRL Evreux. The breeder was CRL France or Italy.

Mean survival rate of male and female SD rats was similar (46% and 42%, respectively), while male and female HW rats survived longer (75% and 64%, respectively).

The most common causes of unscheduled death were tumors (mostly pituitary gland), non-evident cause, forelimb/hindlimb inflammation, skin ulcer/abscess or urogenital inflammation in SD males, tumors (mostly pituitary/mammary glands), non-evident cause or various non-neoplastic lesions in eyes/liver in SD females, tumors (mostly skin), non-evident cause or urogenital inflammation in HW males and tumors (pituitary gland and reproductive tract), non-evident cause, urogenital inflammation or skin ulcer in HW females.

The most common neoplasms originated from tumors in pituitary gland (adenoma; >46%), thyroid gland (C-cell adenoma; >12%) and adrenal gland (benign pheochromocytoma; 8%) in SD males, pituitary gland (adenoma; >72%), mammary gland (fibroadenoma; 41% and adenocarcinoma; 33%), vagina (benign granular cell tumor; >9%) and uterus (endometrial stromal polyp; 8%) in SD females, pituitary gland (adenoma; >23%), mesenteric lymph node (hemangioma; >9%) and thyroid gland (C-cell adenoma; >9%) in HW males and pituitary gland