**P12-36**
**Cytotoxicity of chlorinated flavonoids in osteosarcoma *in vitro* models**

J.M. P. Ferreira de Oliveira[1], C. Proença[1], A. T. Rufino[1], A. M.S. Silva[2], **E. Fernandes[1]**

[1]*LAQV, REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal;*
[2]*LAQV, REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal*

Cancer is the second leading cause of death worldwide, with estimated 9.6 million associated deaths. Osteosarcoma (OS), the most common childhood bone cancer, is treated with a combination of preoperative neoadjuvant therapy, tumor surgical resection and postoperative adjuvant therapy [1]. Nevertheless, as OS therapy is still limited by poor overall survival rates for patients with recurrent OS, novel therapeutic agents are still required. In recent years, several polyphenolic compounds have been described to inhibit several processes related to cancer and to OS in particular [2]. These observations highlight the potential of this type of molecule in OS therapy.

The aim of the present study was to evaluate the *in vitro* cytotoxicity of a group of chlorinated flavonoids in OS. For this, four OS cell lines were incubated with 10, 20, 40, 80 and 160 μM flavonoids with Cl substituents at positions 3, 6, and/or 8 for 48 h, and, subsequently, cell viability was investigated upon incubation with WST-8 reagent, followed by spectrophotometric measurement at 450 nm. Moreover, sulforhodamine B (SRB) assay was used to determine cell growth inhibition, followed by spectrophotometric measurement at 510 nm.

The obtained results suggest that the presence of Cl substituent at C-3 of C-ring improves the cytotoxic activity of the tested flavonoid compounds in OS *in vitro*. These results indicate that 3-chloro-3′,4′,6,7-tetrahydroxyflavone may have an important role on OS therapy, although additional studies are required.

**Acknowledgements**: This work received financial support from the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-029243) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project PTDC/MED-QUI/29243/2017 and from PT national funds (FCT/MCTES) through grant UIDB/50006/2020. ATR and CP thank to FCT for the funding through the project PTDC/MED-QUI/29243/2017. JMPFO thanks FCT for funding through program DL 57/2016 – Norma transitória (ref. SFRH/BPD/74868/2010).

**References**

[1] Anderson M.E., *Orthop. Clin. North Am.* 2016, 47, 283–292.
[2] Ferreira de Oliveira J. M. P., Almeida J. F. D., Martins M., Proença C., Oliveira H., Fernandes E., *Pharmaceuticals (Basel)*. 2021, 14(7), 640.

https://doi.org/10.1016/j.toxlet.2022.07.515

---

**P12–37**
**Investigation of the cytotoxicity of bioceramic nanoparticles on Saos-2 cells by an alternative method**

**A. Tomak,** A. C. Önder, C. Öksel Karakuş

*İzmir Institute of Technology, Department of Bioengineering, İzmir, Turkey*

Cell membrane integrity is frequently assessed as a measure of cell viability and cytotoxic effects. Investigation of nanoparticle-induced cytotoxic effects in relation to the cell membrane is the primary step in the risk assessment of nanoparticles. Although traditional *in vitro* cytotoxicity tests are associated with significantly lower costs compared to *in vivo* approaches, they often require specialized assay kits, which come with their own costs. Moreoever, interference-related issues (e.g. interference of nanoparticles with assay components) may arise when these in conventional cytotoxicity tests are applied to nanoparticles, leading to inaccurate or questionable test results. Therefore, there is a clear need for the development of easy-to-use, accessible, and non-invasive approaches to assess nanoparticle cytotoxicity. Flow cytometry is a well-established tool that allows analysis of multiple parameters of cell death. In relation to nanoparticle toxicity assessment, flow cytometry is commonly used to detect cellular uptake and apoptosis. However, its application to assess cell viability with the use of fluorescent dyes (e.g., propidium iodide (PI) and thiazole orange (TO)) is limited to a few examples in nanotoxicology. Here, we tested the potential use of flow cytometry as an alternative method for monitoring viability of Saos-2 human osteosarcoma cells following exposure to two different bioceramic nanoparticles that are commonly used in dental applications, hydroxyapatite (HAp) and tricalcium phosphate (TCP). First, the bioceramic nanopowders were dispersed in liquid media following modified NANOGENOTOX dispersion protocol. The dispersed nanopowders were characterized by electron microscopy and dynamic light scattering. As a next step, Saos-2 human osteosarcoma cells were treated with varying concentrations of HAp and TCP dispersions for 6 h and 24 h. Cell viability was assessed by tetrazolium-based MTT assay and by means of flow cytometry after staining all cells with TO dye and dead cells with PI dye. We compared the cell viability data obtained by both MTT assay and flow cytometry. The results suggested that flow cytometry can be used as an alternative cell membrane integrity screening method for the detection of injured, dead and viable cells treated with bioceramic nanoparticles.

https://doi.org/10.1016/j.toxlet.2022.07.516

---

**P12-38**
***In vitro* skin irritation of microfibrillated cellulose and silica nanoparticles: sustainable alternatives to developing nanotechnology products**

**J. V. Cruz[2,1],** V. C. Gagosian[1], W. Magalhães[3], P. H. Cademartori[4], C. B. Pestana[1], D. P. de Oliveira[2], D. M. Leme[1]

[1]*Federal University of Paraná, Department of Genetics, Curitiba, Brazil;*
[2]*University of São Paulo, School of pharmaceutical sciences of Ribeirão Preto, Ribeirão Preto, Brazil;*
[3]*Embrapa, Florestas, Colombo, Brazil;*
[4]*Federal University of Paraná, Graduate Program in Engineering and Science of Materials, Curitiba, Brazil*

**Purpose:** Microfibrillated cellulose (MFC) and silica nanoparticles ($SiO_2$NP) have been proposed to develop sustainable and renewable products. MFC presents a thickener property and allows interactions with polymers and other nanoparticles, and $SiO_2$NP can be used as chemical carriers, working as a controlling delivery system. However, nanoparticles may cause toxicity in a pattern different from those observed in their larger physical forms since the nanoscale has increased contact surface and interact differently with biological systems. Therefore, this work aims to verify whether MFC and $SiO_2$NP are skin irritants after acute and repeated exposure using a reconstructed human epidermis (RHE).

**Methods:** The skin irritation test was performed accordingly with OECD TG 439 using an in-house RHE model. The acute exposure of MFC (1%) and $SiO_2$NP (0.5%) followed the SkinEthic protocol. Repeated exposure was performed from an adaptation of the exposure conditions described by the same protocol. For negative control, RHE was exposed to ultrapure water, which was also used as a vehicle. For the positive control, RHE was exposed to sodium dodecyl sulfate at 1%.