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Bioactive compounds and senescence in cell culture models

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SUMMER SCHOOL, Bratislava

Senescent cells

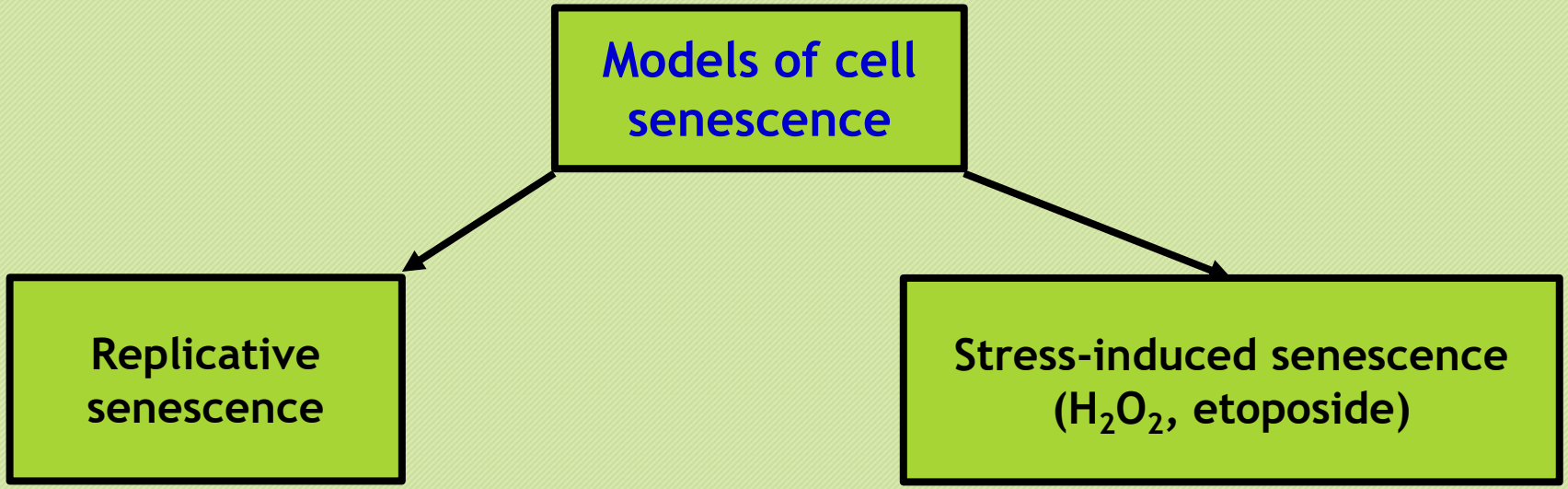
- Removal of senescent cells → increased health span and delayed age-related disorders in a progeroid model of accelerated mouse aging
- Discovery of the first "senolytic" compounds that can selectively target and eliminate senescent cells: Quercetin and Dasatinib

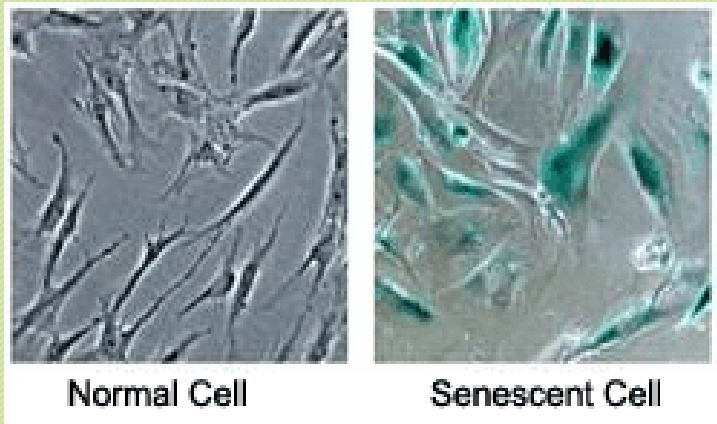
✓ *Baker, C. L., and Pera, M. F. (2018). Capturing totipotent stem cells. Cell Stem Cell 22, 25–34.*

✓ Zhu, Y. et al. (2015). The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 14, 644–658.

Cell culture model of senescence

- **Cell line** - MRC-5 - human lung fibroblasts





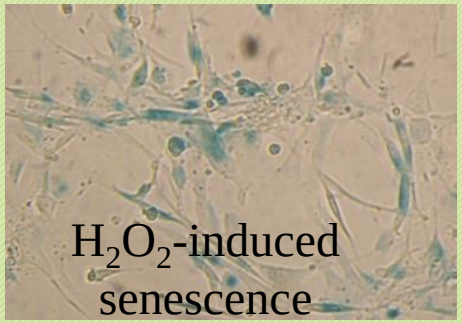
Human MRC-5 fibroblasts
in Petri dish

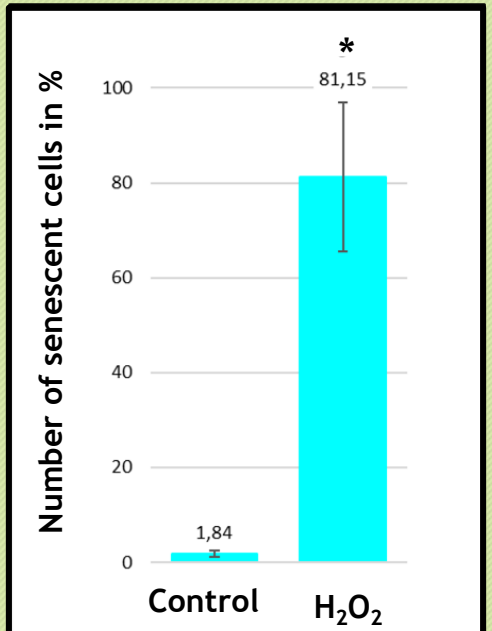
Senescence markers

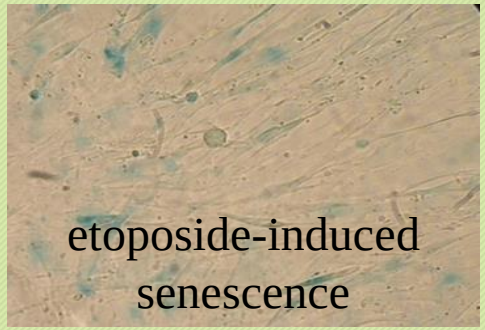
- Increased activity of SA-β-galactosidase
- Decreased cell growth (MTT test)
- Accumulation of cells in the G2/M phase of the cell cycle (flow cytometry)
- Increased expression of p21 and p16 at the protein level (Western blot)
- Increased number of reactive oxygen species

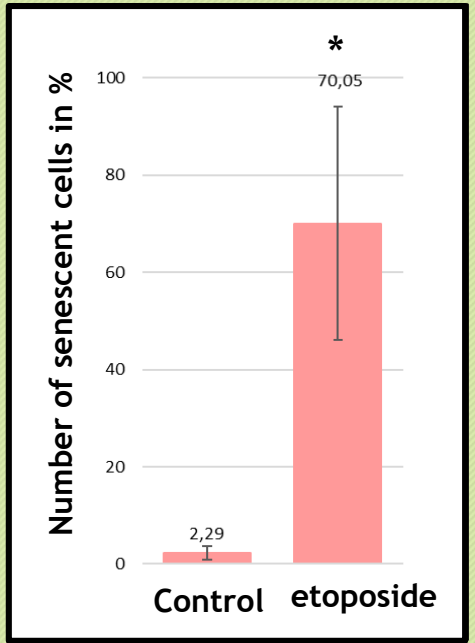
Increased activity of SA-β-galactosidase

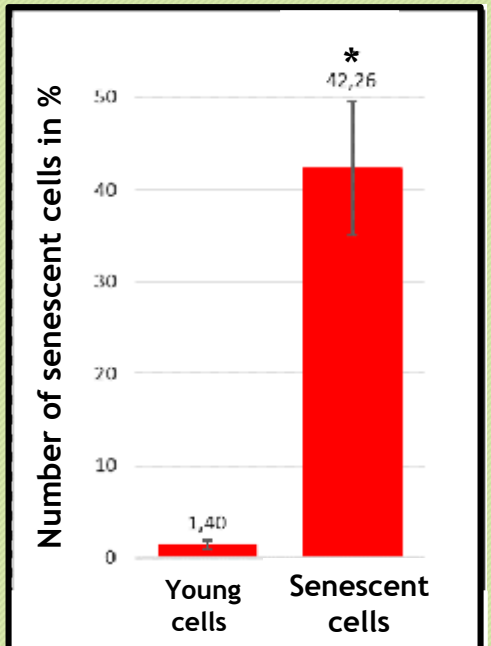
Control











Senescence markers

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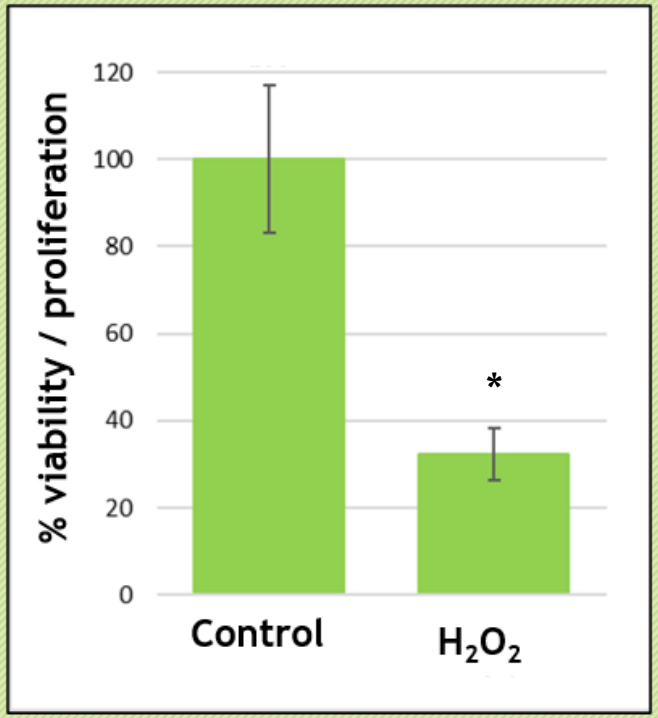
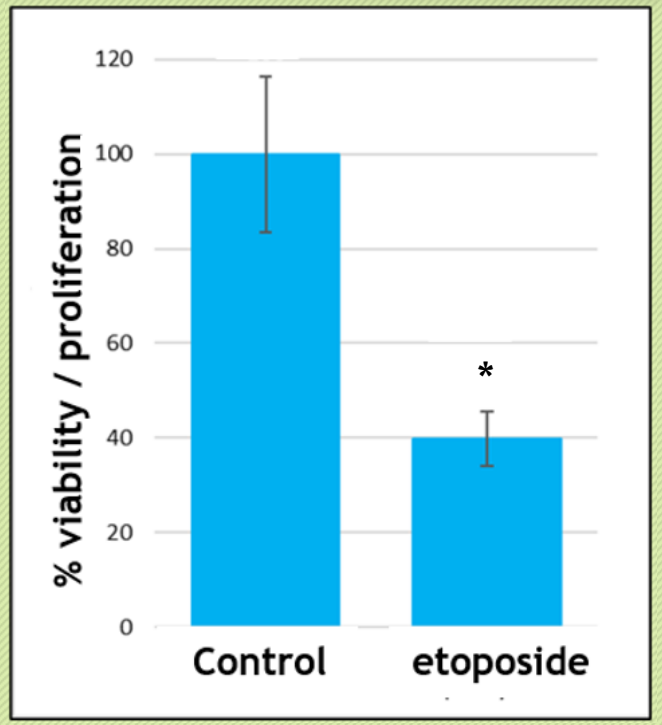
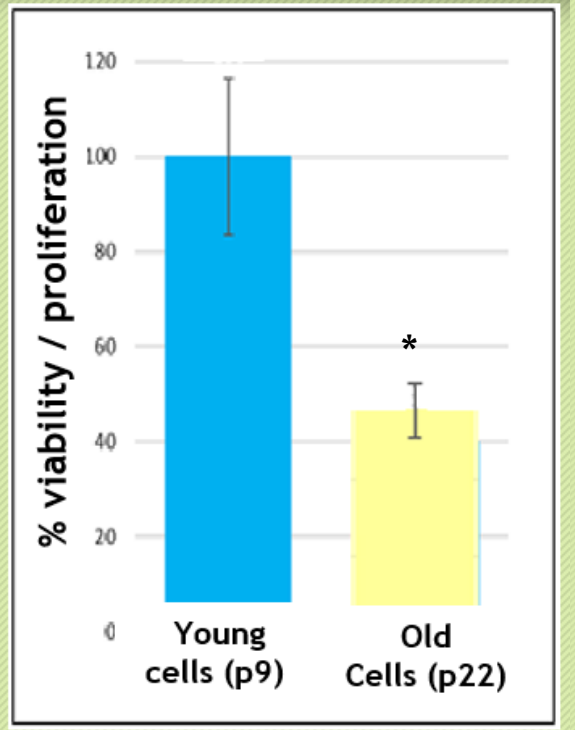
Decreased cell viability/proliferation (MTT test)



H_2O_2-induced senescence



Etoposide-induced senescence



Replicative senescence

Senescence markers

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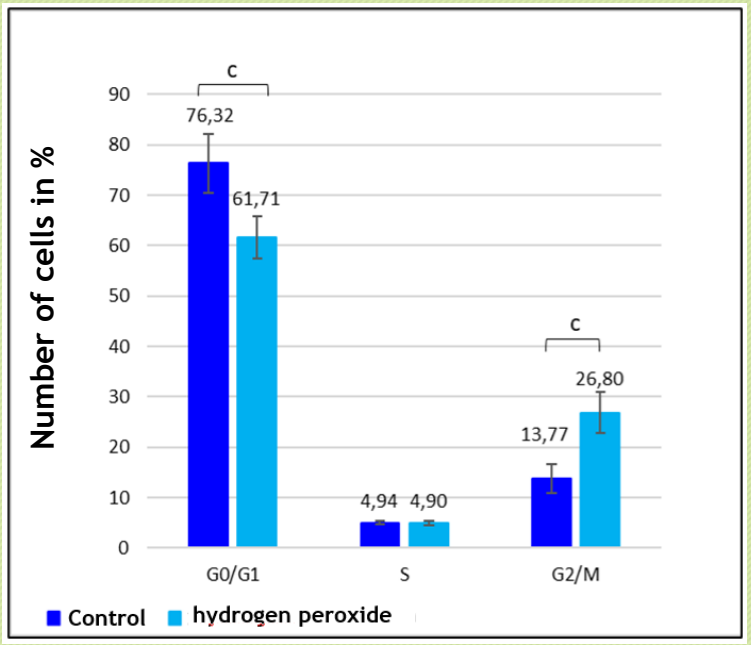
Accumulation of cells in the G2/M phase of the cell cycle (flow cytometry)



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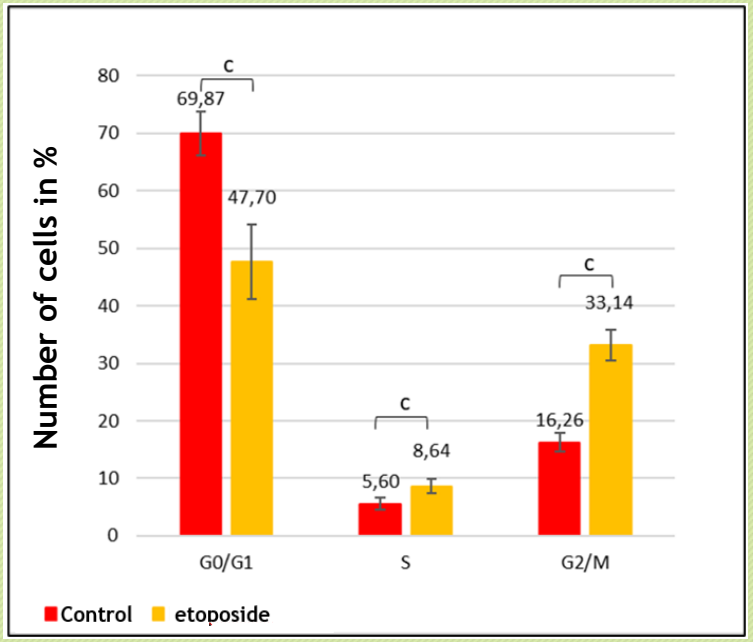
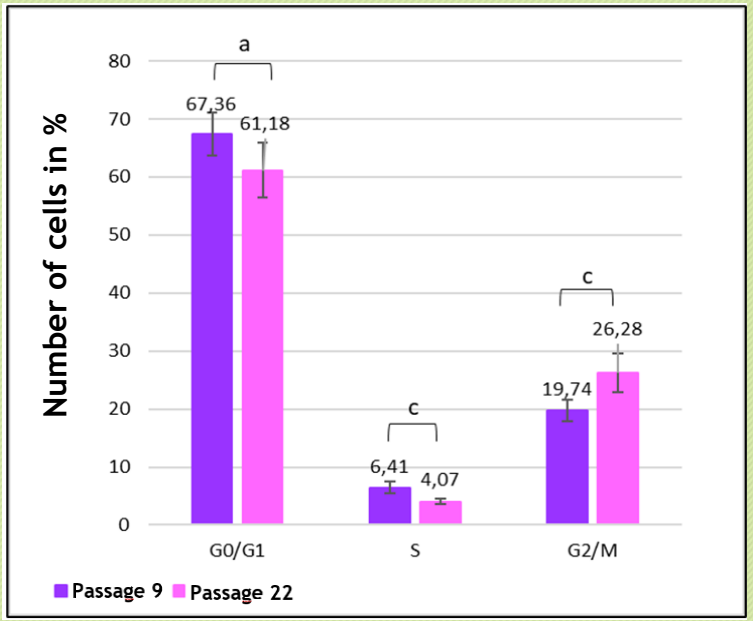


Etoposide-induced senescence



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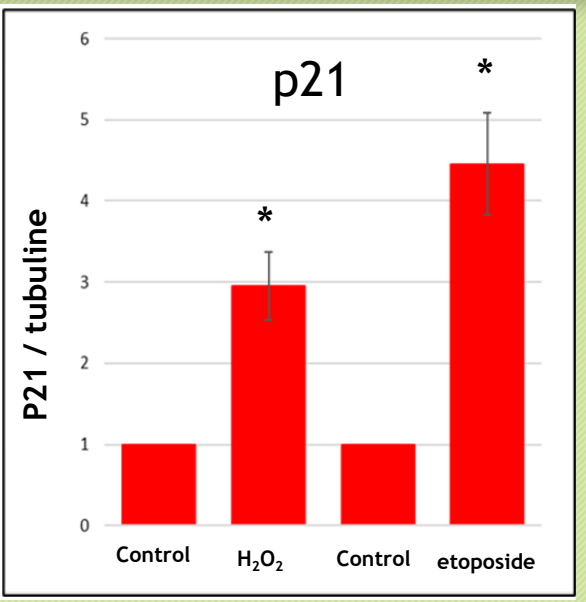
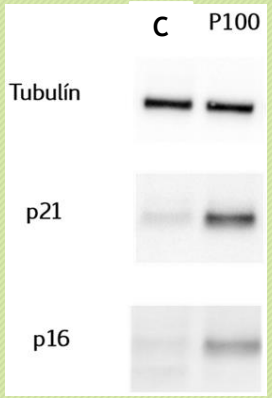
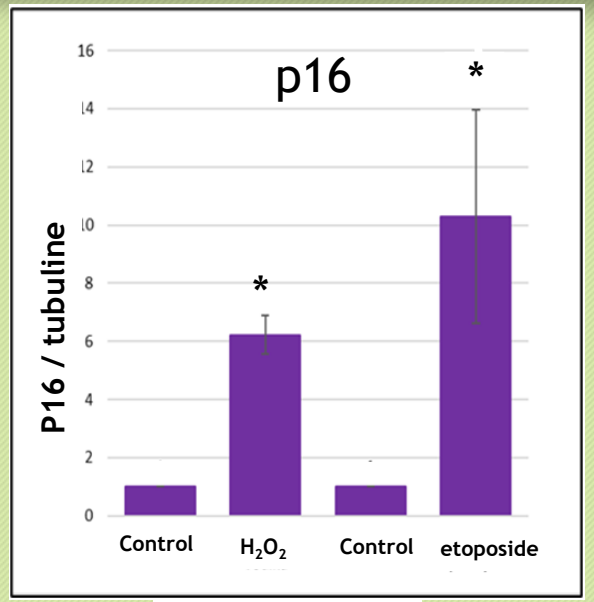
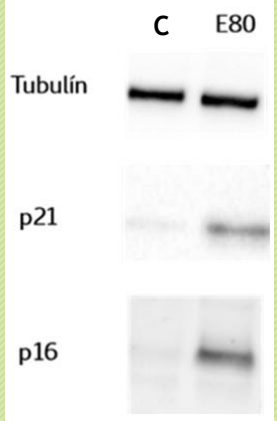
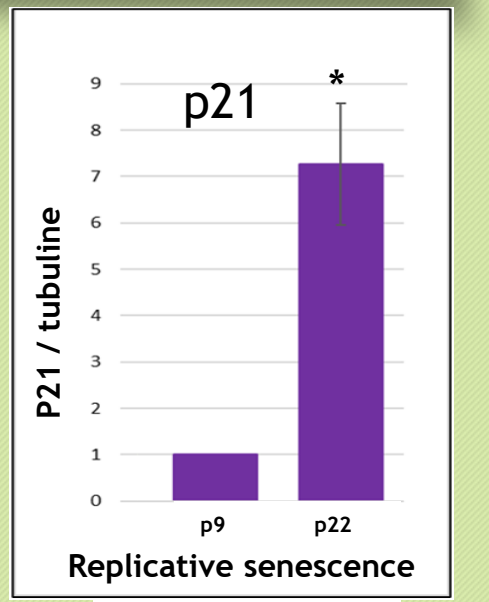
Increased expression of p21 and p16 at the protein level (Western blot)

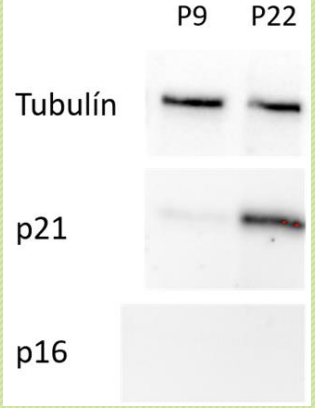












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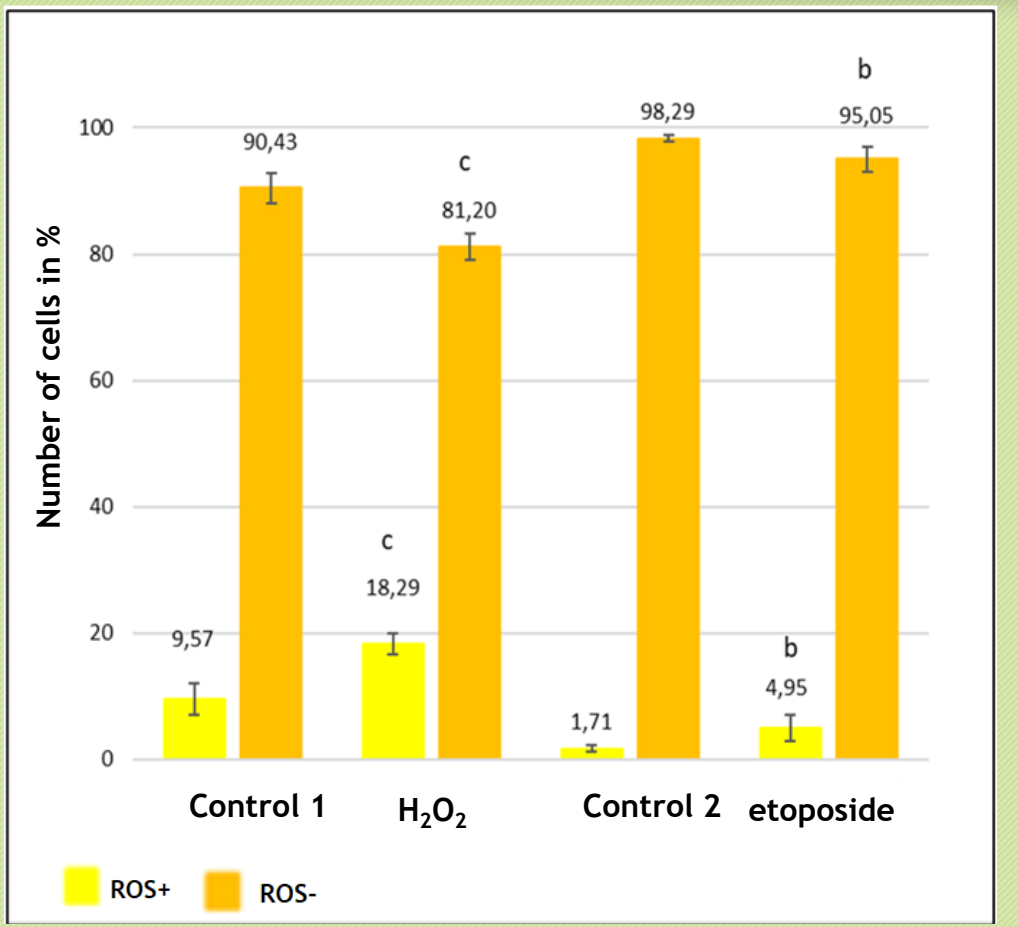
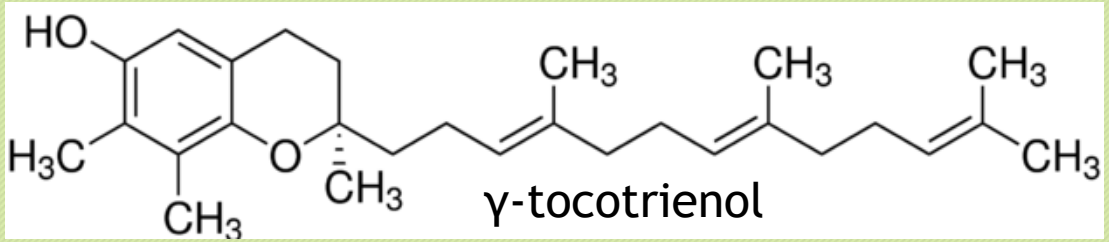
Increased number of reactive oxygen species

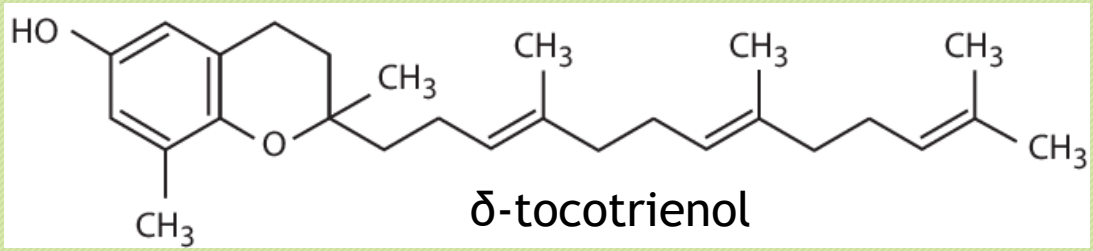


Bioactive compounds

- **γ and δ-tocotrienols**

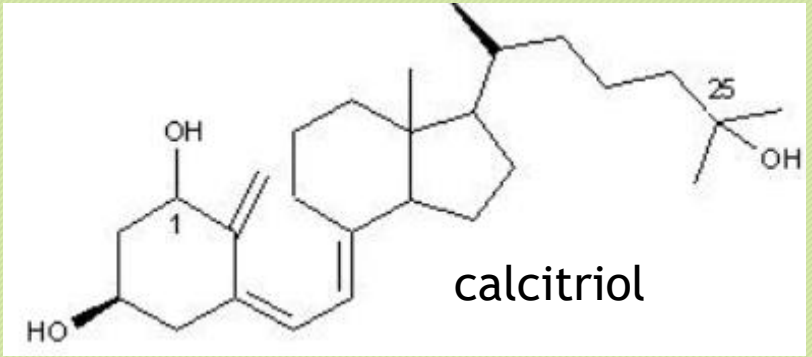


γ-tocotrienol



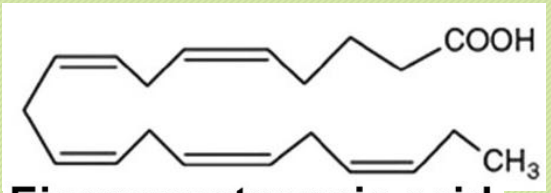
δ-tocotrienol

- **Vitamin D**

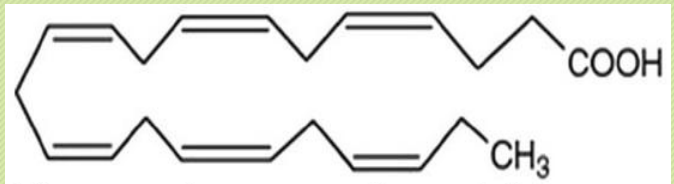


calcitriol

- **Omega-3 fatty acids (EPA and DHA)**



Eicosapentaenoic acid



Docosahexaenoic acid

γ- and δ-tocotrienols

- do not significantly affect the growth of healthy cells
- significantly reduce the cell growth under stressful conditions (H_2O_2, etoposide)
- δ-tocotrienol is more effective than γ-tocotrienol

La Fata, G. et al.(2015). Vitamin E Supplementation Delays Cellular Senescence In Vitro. BioMed Res Int

γ and δ-tocotrienols

Papers:

- ✓ *Janubova M, Zitnanova I: Effects of bioactive compounds on senescence and components of senescence associated secretory phenotypes in vitro. Food Funct. 2017;8(7):2394-2418.*
- ✓ *Janubova M, Hatok J, Konarikova K, Zitnanova I. γ- and δ-Tocotrienols interfere with senescence leading to decreased viability of cells. Mol Cell Biochem. 2021, 476(2):897-908*
- ✓ *Janubová M., Žitňanová I.: Effect of gamma-tocotrienol and delta-tocotrienol on senescent human fibroblats. Spomienka na Ladzianskeho v čase COVID-19. - Bratislava : Univerzita Komenského v Bratislave, 2020,136-141.*

PhD thesis:

- ✓*Janubová M.: Effects of selected bioactive substances on the aging process in a cell culture model. PhD thesis 2020*

Diploma thesis:

- ✓ *Fekete K.: Nutrition and healthy aging. Diploma thesis 2022*

Vitamin D

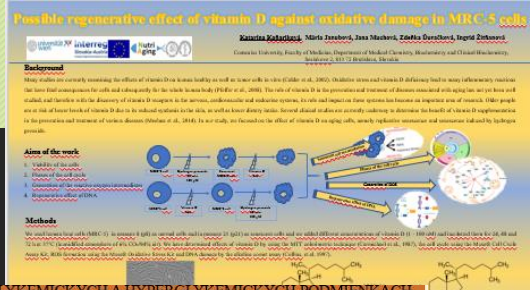
- increases the growth of old cells MRC-5 (passages 21 and 22) compared to young cells (p8, p9)
- reduces the expression of the inhibitor of cell cycle (protein p21)
- increases astrocyte survival in normoglycemic and hyperglycemic conditions

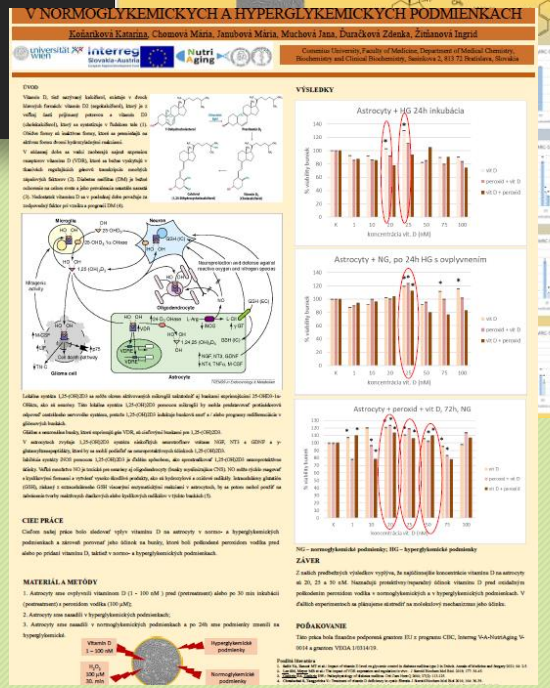
Vitamin D

Papers:

- ✓ *Sedlák M., Žitňanová I.: Effect of vitamin D on metabolism and metabolic syndrome. Anatómia - 100 rokov LF UK. - Trebišov : k-PRINT, 2019. - S. 168-172. - ISBN 978-80-973545-0-3*
- ✓ *Koňariková K., Chomová M., Janubová M., Muchová J., Ďuračková Z., Žitňanová I: Neuroprotective effects of vitamin D. In: Spomienka na Ladzianskeho v čase COVID-19. - Bratislava : UK v Bratislave, 2020.*
- ✓ *Janubová M.: Nenahraditeľná úloha vitamínu D v udržiavaní zdravia. (Irreplaceable role of vitamin D in maintaining health) Šanca 3/2021*
- ✓ *Janubová M., Žitňanová I.: Effect of vitamin D on cell senescence. A stále žijeme v čase Covidu... - Bratislava : UK v Bratislave, 2021. - S. 142-147. - ISBN 978-80-223-5302-1*
- ✓ *Koňariková, K. et al.: Role of Vitamin D in the DNA repair of senescent cells damaged by hydrogen peroxide. Laboratórna diagnostika 1/2021*
- ✓ *Koňariková K.et al. Effects of vitamin D in hyperglycemic conditions. Ladzianskeho zborník – „A stále žijeme v čase covidu...“ - Univerzita Komenského v Bratislave, Bratislava, 2021,148-153 - ISBN 978-80-223-5302-1*

Vitamin D





Diploma thesis:

- ✓ *Sedlák M. Effect of bioactive substances on the development of age-associated diseases. Diploma thesis 2018*

Conferences:

- ✓ *Koňariková K., Janubová M., Muchová J., Ďuračková Z., Žitňanová I.: Possible regenerative effect of vitamin D against oxidative damage and subsequent tumor growth in human lung cancer cells. Conference in Prague, 2021*
- ✓ *Koňariková K., Chomová M., Janubová M., Muchová J., Ďuračková Z., Žitňanová I: Biological effect of vitamin D on astrocyte viability in normoglycemic and hyperglycemic conditions. Scientific-pedagogical conference of teachers of biochemistry departments of medical faculties in the SR and CR, Martin, 16.6.-19.6.2022*

Omega-3 FA: EPA and DHA

- Increase the cell growth of lung fibroblast MRC-5 cells
- DHA is more effective than EPA
- Inhibit development of senescence by reducing:
  - ✓ the number of cells that show increased SA-ß-galactosidase activity
  - ✓ the expression of cell cycle inhibitor – protein p21
  - ✓ the number of reactive oxygen species

Omega-3 FA

Papers:

- ✓ *Sedlák, M., Žitňanová, I.: Omega-3 fatty acids and cardiovascular diseases. In: Spomienka na Ladzianskeho v čase COVID-19. - Bratislava : UK v Bratislave, 2020.*
- ✓ *Janubová M., Gbelcová H., Koňariková K., Szentešiová Z. and Žitňanová, I.: DHA and EPA are Able to Affect the Development of Stress-Induced Senescence. Austin Med Sci. 2022; 7(1): 1062.*

Diploma thesis:

- ✓ *Teresa Ridder: Proteins and omega-3 fatty acids: health benefits during aging. Diploma thesis 2021*

Conferences:

- ✓ *Janubová M., Koňariková K., Žitňanová I.: Effect of omega-3 fatty acids on stress-induced senescence. XII. year of the Interactive Conference of Young Scientists 2020. Bratislava, May 7 - June 5, 2020*
- ✓ *Janubová M., Koňariková K., Žitňanová I.:DHA and EPA affect development of stress-induced premature senescence. Scientific-pedagogical conference of teachers of biochemistry departments of medical faculties in the SR and CR, Martin, 16.6.-19.6.2022*

General papers on senescence

- ✓ *Pazderová Petra: Role of vitamin c in healthy aging. Šanca 2/2022*
- ✓ *Janubová Mária, Žitňanová Ingrid: Effect of some vitamins on cell senescence. A stále žijeme v čase Covidu... - Bratislava : UK v Bratislave, 2021, 136-142. - ISBN 978-80-223-5302-1*
- ✓ *Žitňanová Ingrid: Can antioxidants protect us from age-related diseases? Šanca 1/2021*
- ✓ *Žitňanová Ingrid: Oxidative stress and antioxidants. Seniors.sk, 14. apríla 2021*
- ✓ *Žitňanová Ingrid, Oravec Stanislav, Janubová Mária et al.: Gender differences in LDL and HDL subfractions in atherogenic and nonatherogenic phenotypes. Clinical Biochemistry. - 79 (2020), 9-13*
- ✓ *Janubová Mária, Žitňanová, Ingrid: Vplyv etanolových hubových extraktov G. lucidum a H. erinaceum na rast mladých a starých buniek. Horizonty anatómie. - Bratislava : Proprint, 2018, 160-167. - ISBN 978-80-89747-10-8*

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PhD-, Diploma students

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Interreg
Slovakia-Austria
European Regional Development Fund

Interreg Slovakia – Austria