An investigation towards a mitochondria-targeted therapy

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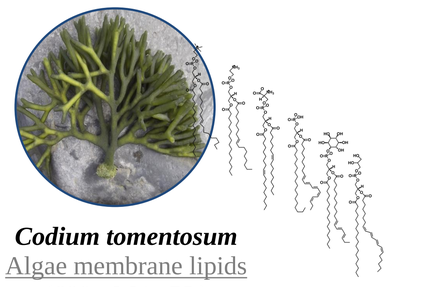
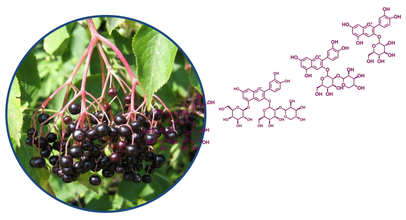
BACKGROUND:

The development of therapeutic strategies to modulate the mitochondrial function is a great scientific challenge, since mitochondrial dysfunction is a common pathological hallmark of many chronic diseases, including degenerative brain pathologies like Parkinson's (PD) and Alzheimer's (AD) diseases. In the present work, this challenge is addressed with an innovative nanophytosomes formulation, engineered with algae membrane polar lipids, obtained from *Codium tomentosum* and elderberry anthocyanins-enriched extract from *Sambucus nigra*. This nanophytosomes formulation is nanosized vesicles with a strongly negative surface charge that preserve the mitochondriotropic properties of elderberry anthocyanins [1].

EXPERIMENTAL DESIGN:



Codium tomentosum
Algae membrane lipids
(600 µM in PL)

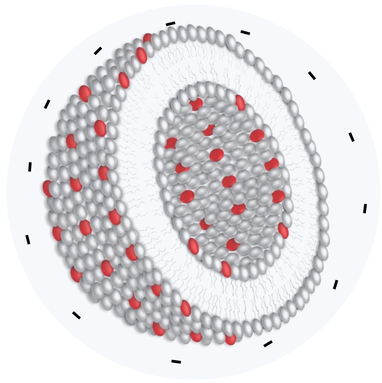


***Sambucus nigra* L.**
Anthocyanins-enriched extract
(0.25 mg/mL)

NANOPHYTOSOMES FORMULATION

Zeta potential: -46.93 ± 6.63 mV

Size: 108.74 ± 24.74 nm



In vitro effects

In vivo/ex vivo effects

Human neuronal SH-SY5Y cells

TOXICITY/SAFETY PROFILE	Cell viability - MTT reduction assay
MITOCHONDRIAL FUNCTIONALITY	Effects on mitochondrial potential and on complexes I and II activity
CELLULAR UPTAKE	Green-fluorescent labelled nanophytosomes

Rotenone-induced rat model of Parkinson's disease (oral administration)

MOTOR AND BALANCE COORDINATION	Beam walking test
BRAIN MITOCHONDRIA FUNCTIONALITY	Basal ganglia, substantia nigra plus cerebellum mitochondrial complexes I, II and IV activity

Nanophytosomes were mainly internalized by cells *via* caveola-mediated endocytosis and have the competence to target mitochondria, improving the mitochondrial respiratory chain complexes I and II and preserving the mitochondrial membrane potential in the presence of rotenone

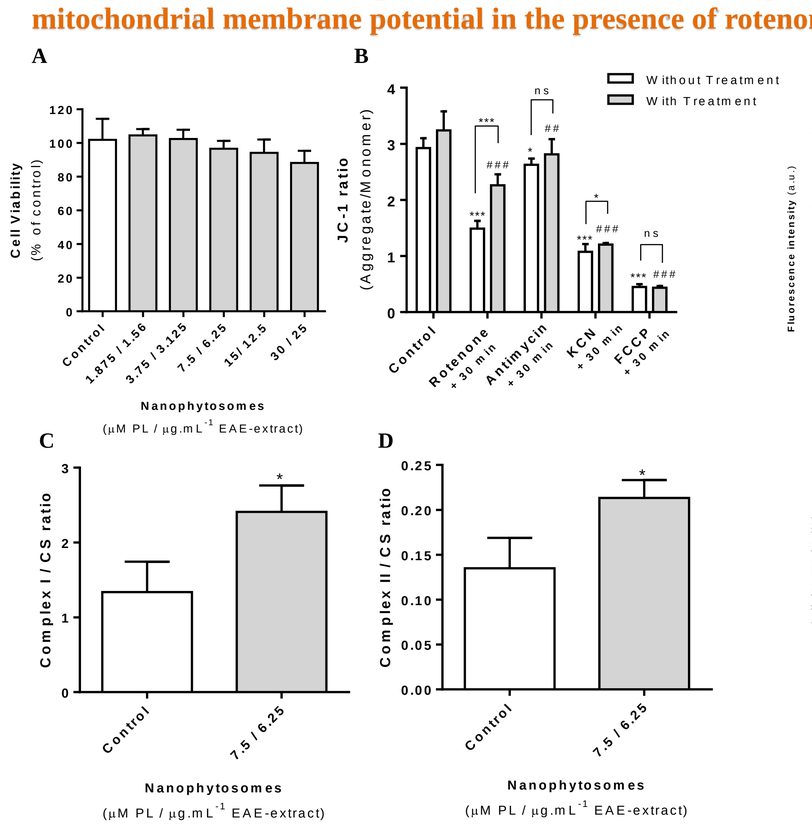


Figure 1. Effects of increasing concentrations of nanophytosomes on the viability of SH-SY5Y cells, assessed by the MTT reduction assay after 24 h of incubation (A). Effects of the treatment of SH-SY5Y cells with nanophytosomes (7.5 µM PL/6.25 µg. mL-1 EAE-extract) on the mitochondrial membrane potential sensitivity to rotenone, antimycin A, KCN and FCCP, assessed by JC-1 probe. Results are expressed by the ratio of red (aggregate) to green (monomer) fluorescence JC-1 (B) . Effects of the treatment of SH-SY5Y cells with nanophytosomes (7.5 µM PL/6.25 µg. mL-1 EAE-extract) on the activity of mitochondrial complex I (C) and complex II (D) normalized by citrate synthase. *,*** Significantly different from control condition, with $p \leq 0.05$ and $p < 0.001$, respectively. ##,### Significantly different from the matched untreated cells, with $p < 0.01$ and $p < 0.001$, respectively; n.s without significant differences.

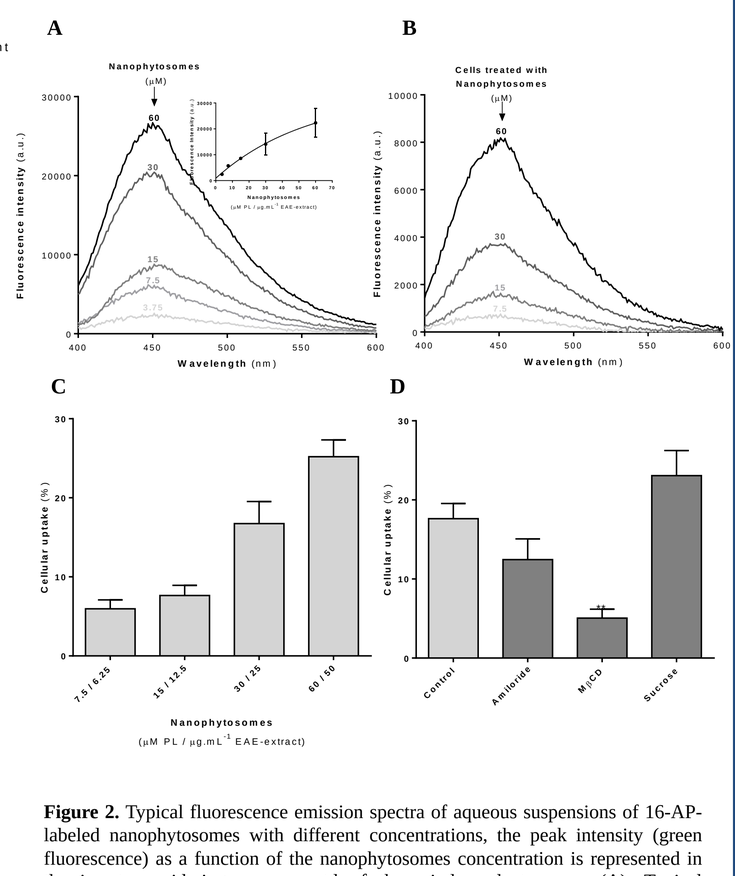


Figure 2. Typical fluorescence emission spectra of aqueous suspensions of 16-AP-labeled nanophytosomes with different concentrations, the peak intensity (green fluorescence) as a function of the nanophytosomes concentration is represented in the insert considering mean ± std of three independent assays (A). Typical fluorescence emission spectra of SH-SY5Y cells exposed at different concentrations of 16-AP-labeled nanophytosomes for 2 h. All emission spectra were obtained, setting the excitation wavelength at 365 nm (B). Quantification of cellular uptake of green-fluorescent labelled nanophytosomes in SH-SY5Y cells exposed at different concentrations for 2 h (C). Effects of the SH-SY5Y cells pre-treatment with amiloride, methyl-β-cyclodextrin (MβCD) or sucrose (endocytosis inhibitors) for 30 minutes on the cellular uptake of nanophytosomes (30 µM PL/25 µg. mL-1 EAE-extract) (D). ** Significantly different from control condition, with $p < 0.01$.

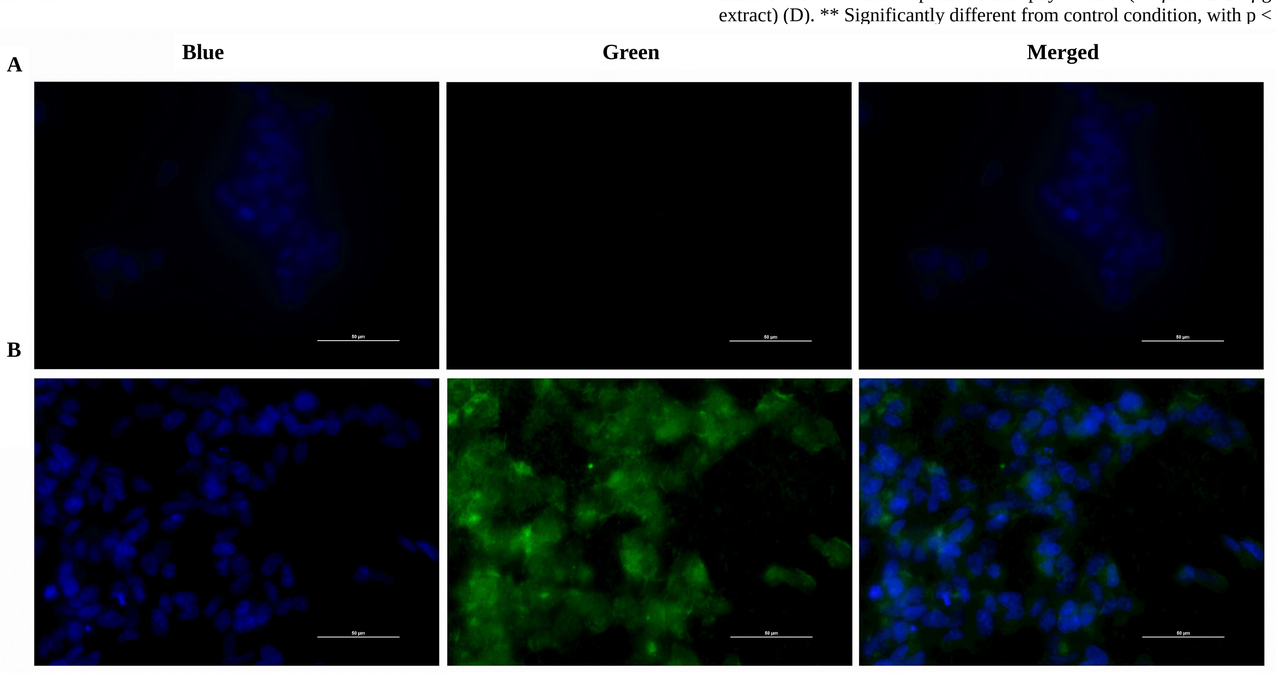


Figure 3. Fluorescence microscope images of control (A) and green-fluorescent labelled nanophytosomes treated (B) SH-SY5Y cells. Hoechst was used to stain the nuclei of the cells of both conditions.

Oral administration of Nanophytosomes improves the motor coordination and balance disturbance of Rotenone-induced rat model of Parkinson's disease

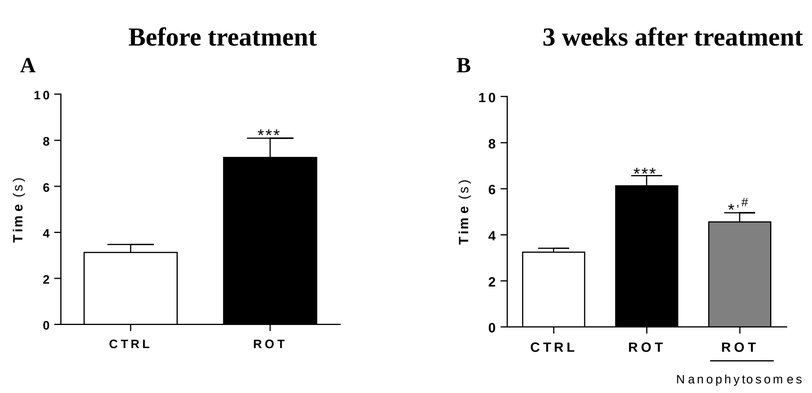


Figure 4. Average times for crossing the 6 mm beam in male Wistar rats control and pretreated with rotenone (3.0 mg/kg, i.p.) (A). Effect of treatment with nanophytosomes for 3 weeks in average times for crossing the 6 mm beam in groups untreated and treated (B). **, *** Significantly different from control condition, with $p < 0.01$ and $p < 0.001$, respectively. # Significantly different from the matched untreated cells, with $p \leq 0.05$ respectively.

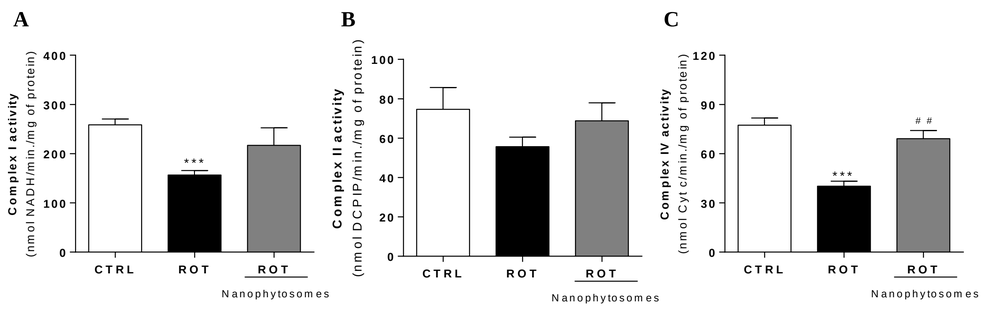


Figure 5. Effects of treatment with nanophytosomes on the activity of mitochondrial complex I (A), complex II (B) and complex IV (C) evaluated in mitochondria-enriched fraction obtained from basal ganglia, substantia nigra plus cerebellum of untreated and treated animals with nanophytosomes for 3 weeks. *** Significantly different from control condition, with $p < 0.001$, respectively. ## Significantly different from the matched untreated cells, with $p < 0.01$.

CONCLUSIONS:

Cellular assays demonstrated the non-cytotoxicity of the nanophytosomes and confirmed their competence to target mitochondria mainly internalized *via* caveola-mediated endocytosis. Thus, the nanophytosomes enhance the elderberry anthocyanins' ability to modulate mitochondria functionality, improving the activity of mitochondrial complexes I and II and ensuring the $\Delta\Psi m$ in the presence of a complex I inhibitor. The therapeutic efficacy of the oral administration of this nanophytosomes formulations is being tested in rat with PD-like pathology. Our preliminary results indicate that the nanophytosomes promote positive outcomes on the disabling motor symptoms, as well as on other biochemical pathological hallmarks exhibited by PD animal model, suggesting that they have competence to reach the brain and improve the functionality of the brain cells. Overall, data indicate that nanophytosomes have potential to support a mitochondria-targeted therapy for neurodegenerative diseases.

REFERENCES:

[1] Neves et al (2019), *Journal of Functional Foods* 56:145-155

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