Title: Neuroprotective effect of polyphenol enriched fractions on the rotenone-induced neurodegeneration model of Parkinson Disease

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Introduction

Neurodegeneration in Parkinson's and other neurodegenerative diseases involves a complex set of oxidative reactions leading to neuronal death. A wide array of polyphenols has been reported to have substantial neuroprotective activity. These effects have been attributed to their general free radical trapping per se, on neurons, but they also intervene in multiple biological processes, such as iron chelation, activation of cellular stress–response pathways and survival genes, cell signaling pathways and regulation of mitochondrial function.

Pesticide exposure is epidemiologically associated with PD, and administration of the organic pesticide rotenone to rats recapitulates most of the PD symptomatology. In this work we will implement a cell culture model of PD: a neuroblastoma cell line will be exposed to rotenone, a pesticide which specifically inhibits the mitochondrial electron transport chain at complex I; this model was selected because it reproduces *in vitro* the accumulation of the cytoplasmic inclusions, a feature that other neurotoxin models fail to reproduce. These cells will be chronically exposed to the neurotoxin rotenone, a treatment that induces most features of PD, including degeneration of the nigrostriatal pathway and formation of alpha-synuclein-positive cytoplasmic inclusions, termed *Lewis bodies.*

In this project we intend to evaluate the neuroprotective effect of some selected polyphenol enriched fractions from Portuguese endemic plants from genera *Juniperus* and *Rubus* on the rotenone-induced neurodegeneration model of Parkinson Disease

Working plan:

Task 1 Implementation of the rotenone-induced neurodegeneration cell model of Parkinson's disease (PD). SK-N-MC cells will be grown during four-week in two different media: medium only and medium supplemented with 5 nM rotenone. Cell viability will be followed during the 4 week treatment by a fluorescent method and induction of oxidative stress by flow cytometry using a probe specific for ROS.

Task 2- Analysis of cytotoxicity, antioxidant and neuroprotective capacities for polyphenol enriched fractions. Chemical screening for antioxidant properties in Portuguese endemic plants already performed in Disease and Stress Biology laboratory lead to the selection of genera *Juniperus* and *Rubus* as promising sources of antioxidant phytochemicals. The cytotoxicity of the selected polyphenol enriched fractions will be screened by a cell viability assay in SK-N-MC cells in order to identify the nontoxic range. Afterwards, the biological activity of the selected phenolic enriched fractions will be further characterized using a more specific antioxidant bioactivity assay performed on oxidative stress induced neuroblastoma cells. The aim herein is to evaluate the intracellular antioxidant activity and thus neuroprotective of these fractions, monitoring the formation of intracellular reactive oxygen species by flow cytometry.

Task 3 – Study of the effect of chronic exposure to polyphenol enriched fractions on the oxidative stress status of the Parkinson induced cells. After confirmation that the chronic exposure of cells to the optimized concentrations of polyphenol enriched fractions is nontoxic we will evaluate the putative protective role of the selected fractions on the neurodegeneration model of Parkinson Disease by flow cytometry using a probe specific for ROS.