

## **Acteoside exerts neuroprotective effects by preventing $\alpha$ -synuclein aggregation and oxidative stress**

Giorgia Spandri<sup>1</sup>, Alessia Lambiase<sup>1,2</sup>, Hind Moukham<sup>2</sup>, Elisa Toini<sup>1,2</sup>, Giovanni Zecca<sup>1,2</sup>, Carlo Santambrogio<sup>1</sup>, Maura Brioschi<sup>1</sup>, Fabrizio Grassi<sup>1,2</sup>, Farida Tripodi<sup>1,2</sup>, Paola Coccetti<sup>1,2</sup>, Massimo Labra<sup>1,2</sup>

<sup>1</sup> Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy.

<sup>2</sup> National Biodiversity Future Center (NBFC), Palermo, Italy.

$\alpha$ -Synuclein is a small presynaptic protein whose aggregation is one of the hallmarks of Parkinson's disease (PD), a neurological disorder that affects 10 people worldwide. In our quest to identify novel preventive or therapeutic treatments for PD, and following the bioprospecting research approach, we collected 60 Italian plant species to identify new neuroprotective bioactive compounds.

Through a high-throughput screening on yeast cells expressing human  $\alpha$ -synuclein, combined with an *in silico* phylogenetic analysis, we identified *Verbascum thapsus* as potential neuroprotective and antiaggregant plant. Its extract exhibits robust inhibitory activity against the amyloid aggregation of  $\alpha$ -synuclein *in vitro*, as well as in neuroblastoma cells overexpressing the protein, where  $\alpha$ -synuclein oligomers were strongly reduced.

By employing a size exclusion chromatography affinity approach coupled to mass spectrometry (in collaboration with Prof. Enrica Calleri, University of Pavia), we identified the phenylpropanoid glycoside acteoside from the extract of *V. thapsus* as the metabolite that directly binds  $\alpha$ -synuclein. This binding was also confirmed by native-MS. Through this interaction, acteoside inhibits both primary nucleation, by preventing nuclei formation during the lag phase, and fibril-induced amplification during the growth phase of  $\alpha$ -synuclein fibrils, indicating it serves as a strong inhibitor of protein aggregation at multiple stages of the process.

In addition, acteoside reduces oxidative stress in neuroblastoma cells exposed to  $\alpha$ -synuclein fibrils and activates the NRF2 pathway, as shown by the increased nuclear localization of NRF2 and increased mRNA level of NRF2-dependent genes in cells treated with acteoside.

Notably, acteoside improves motor performance in a *Drosophila* model of PD and exhibits a significant reduction of protein carbonyl groups, suggesting that this compound may mitigate oxidative stress-induced protein damage also in an *in vivo* model of PD.

Altogether, our findings could pave the way for the development of new strategies aimed at developing acteoside as a novel neuroprotective agents targeting PD.