Isolation and characterization of natural bioactive polyphenols with antioxidant and anti-Prion properties

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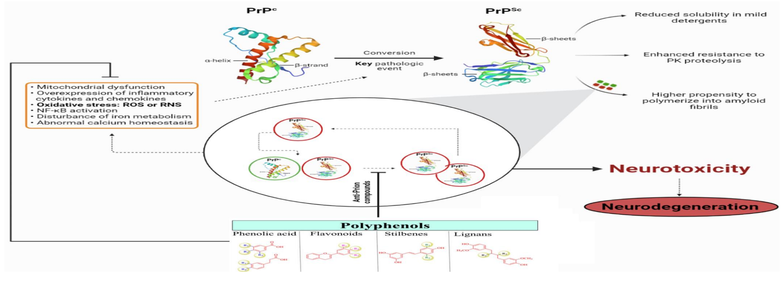
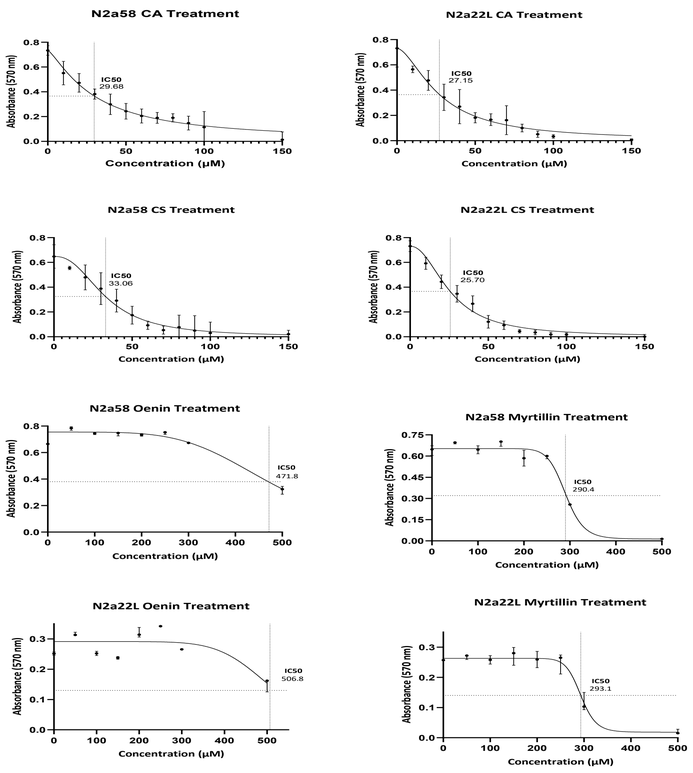
Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of rare, fatal brain diseases that affect both animals and humans - and are caused by the misfolding of normal protein (PrP^{C}) into disease-associated protein (PrP^{Sc}). Oxidative stress has been found to be associated with the onset or worsening of neurodegenerative diseases. The polyphenols are naturally occurring compounds and have the ability to act as inhibitors of oxidative stress and can therefore exploit as antioxidants and were tested for their antiprion effects.



Results

Toxicity levels of Carnosic Acid and Carnosol are particularly higher than Oenin and Myrtillin



Carnosic acid (CA):
- N2a58 IC_{50}: 29.68μM
- N2a22L IC_{50}: 27.15μM

Carnosol (CS):
- N2a58 IC_{50}: 33.06μM
- N2a22L IC_{50}: 25.70μM

Oenin:
- N2a58 IC_{50}: 471.80μM
- N2a22L IC_{50}: 506.80μM

Myrtillin:
- N2a58 IC_{50}: 290.4μM
- N2a22L IC_{50}: 293.1μM

Figure 1. Cell viability assessment by means of the 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for Carnosic acid, Carnosol, Oenin and Myrtillin following 48 h incubation in N2a58 and N2a22L cells.
Graphs depict the absorbance at 570 nm determined for each cell line following treatment with the indicated compounds at different concentrations. The background absorbance of the plates at 630 nm was also measured and subtracted from 570 nm measurement. LD_{50} was estimated for each compound and cell line based on non-linear regression analysis for curve fitting using the GraphPad software (v 8.0). The black dots in graphs for Polyphenols represent the fitted curve in each case; determined LD_{50} values are depicted in each graph.

Production of ROS was significantly decreased in the presence of Polyphenols for both cell lines

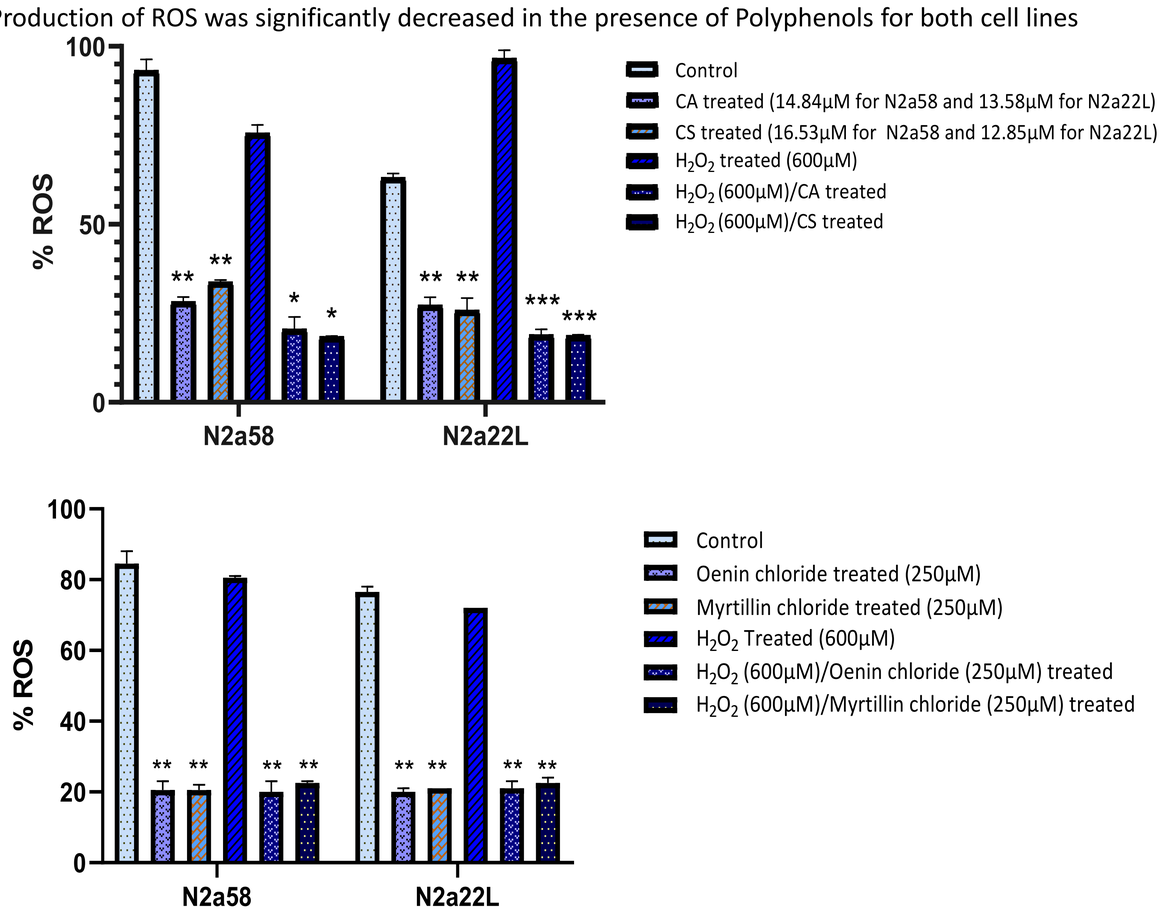


Figure 2. Reactive oxygen species (ROS) measured using H_2DCFDA in N2a58 and N2a22L cell lines after treatment with the 0.5 × LD50 of Polyphenols without or following pre-treatment with 600 μM H_2O_2.
The % ROS was calculated based on the maximum ROS production value (3 mM). Data represent means ± SD; stars denote statistical significance (unpaired, one-tailed, *T*-test); *: *p*-value < 0.05, **: *p*-value < 0.01, ***: *p*-value < 0.001.

Treatment upregulates PRNP expression

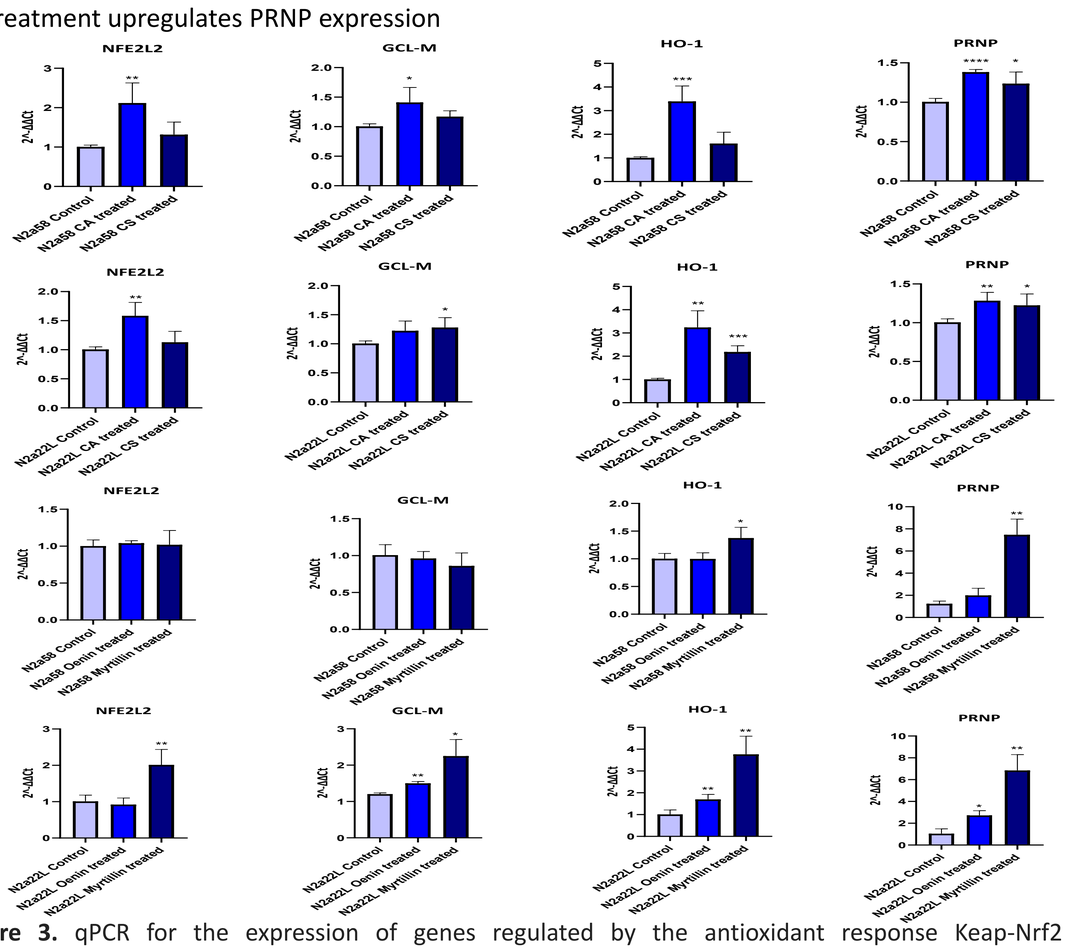


Figure 3. qPCR for the expression of genes regulated by the antioxidant response Keap-Nrf2 pathway and *PRNP* was performed using mRNA extracted from N2a58 and N2a22L cells treated with 0.5 × LD_{50} of Polyphenols for 48 h and compared to non-treated control cells (Cntrl).
Data represent means ± SD of three independent experiments. Stars denote statistical significance (unpaired, one-tailed, *T*-test); *: *p*-value < 0.05, **: *p*-value <0.01, ***: *p*-value < 0.001, ****: *p*-value < 0.0001.

Western-blot of CA- and CS- treated N2a22L cells validated the prevention of PrP^{Sc} formation

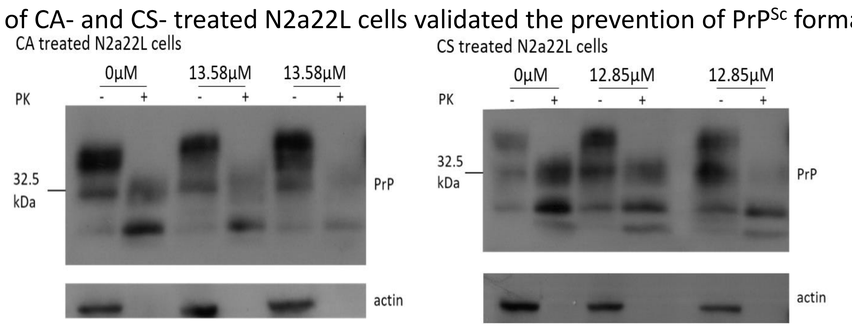


Figure 4. Effect of Carnosic acid and Carnosol treatment on PrP^{Sc} levels (in vitro assays). N2a22L cells were incubated with 0.5 × LD_{50} of CA and CS for 48 h or left untreated (Control), lysed, and then divided into two different fractions. Fraction lysates were either treated (+) or not (-) with PK, and 50 μg of PK-untreated or 100 μg of PK-treated fraction was resolved by SDS-PAGE and immunoblotted with the anti-PrP antibody 6H4 (1:5000) and an anti-β-actin (1:2000) antibody.

In RT-QuIC assays Polyphenols prevented the conversion of $recPrP^{C}$ to PrP^{Sc} and disrupted the already formed aggregates

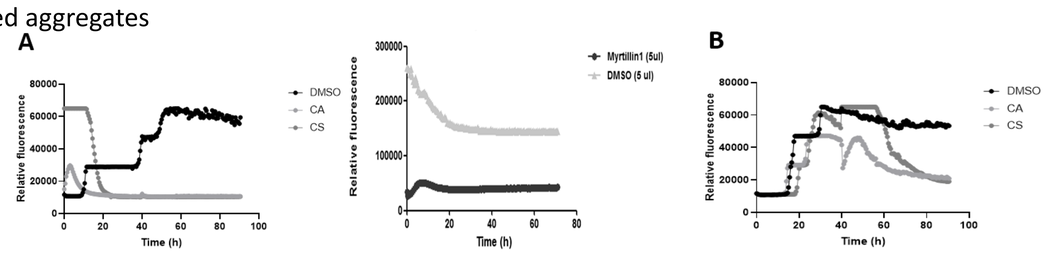


Figure 5. RT-QuIC assays. Aggregation of recPrPC in RT-QuIC assays using CSF from four different sCJD patients as a seed was evaluated in the presence or absence of Carnosic acid, Carnosol and Myrtillin (1 mM final concentration). A) Carnosic acid, Carnosol and Myrtillin were added to the reactions from the beginning, and the Th-T fluorescence was recorded every 30 min. In control reactions containing only DMSO, the solvent vehicle for the compounds, PrPSc aggregates are formed, whereas CA, CS and Myrtillin block their formation. (B) RT-QuIC assays were started without the addition of the compounds, and Th-T fluorescence reached a plateau, indicating conversion and aggregation of PrPSc. The addition of CA and CS to the reaction mixture led to a pronounced decline in Th-T fluorescence.

Conclusions

Our findings suggest that polyphenols increase anti-oxidant response, and they have pleiotropic effects against Prion diseases, suggesting that they could become important preventative and/or therapeutic agents against Prion and other neurodegenerative diseases.

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