# Anthocyanins Display Antioxidant and Anti-Prion Properties in In Vitro Models of Prion Diseases


**Nikoletta Christoudia[1], Arketa Mesi[1], Georgios Alexandros Charisopoulos[1] , Ioannis Tsamesidis[3], Matthias Schmitz[4], Athanasia Chatziefstathiou[1], Eirini Kanata[2], Dimitra Dafou[1], Konstantinos Xanthopoulos[2], Theodoros Sklaviadis[2]**

*[1] Department of Genetics, Development, and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*

*[2] Neurodegenerative Diseases Research Group, Department of Pharmacy, School of Health Sciences, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*

*[3] Department of Prosthodontics, School of Dentistry, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece*

*[4] Department of Neurology, German Center for Neurodegenerative Diseases (DZNE), University Medicine Göttingen 37075, Göttingen, Germany*


Ελληνική Εταιρεία Βιολογικών Επιστημών

## Introduction

Prion diseases, also known as Transmissible Spongiform Encephalopathies (TSEs), refer to a group of rare, progressive neurodegenerative disorders that affect both humans and animals ; and are caused by the misfolding of the normal $PrP^{C}$ protein into a disease-associated pathogenic isoform, $PrP^{Sc}$. Oxidative stress has been found to be associated with the onset or worsening of neurodegenerative diseases. Anthocyanins are characterized as water-soluble bioactive compounds and have the ability to act as inhibitors of oxidative stress, and can therefore exploit as antioxidants.

## Results

### Toxicity Levels of Myrtillin Chloride is partially higher than Oenin Chloride for N2a58 and N2a22L Cell Lines

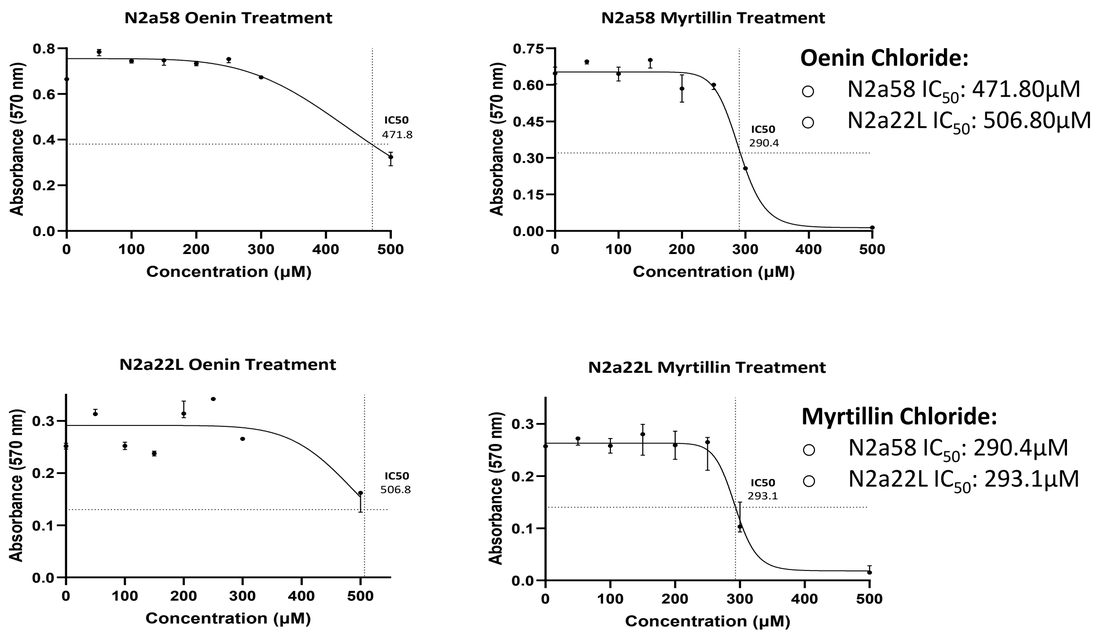


**Figure 1.** Cell viability assessment by means of the 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for Oenin Chloride and Myrtillin Chloride following 48 h incubation in N2a58 and N2a22L cells.

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 Anthocyanins represent the fitted curve in each case; determined $LD_{50}$ values are depicted in each graph.

### Production of Reactive Oxygen Species (ROS) decreased in the presence of Anthocyanins for both cell lines

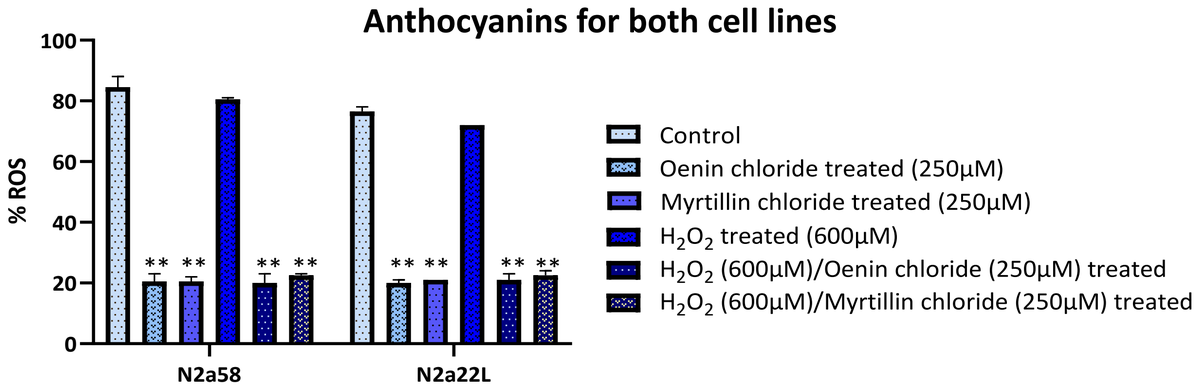


**Figure 2.** Reactive Oxygen Species (ROS) measured using $H_2$DCFDA in N2a58 and N2a22L cell lines after treatment with the 0.5 × LD50 of Anthocyanins without or following pre-treatment with 600 μM $H_2O_2$.

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

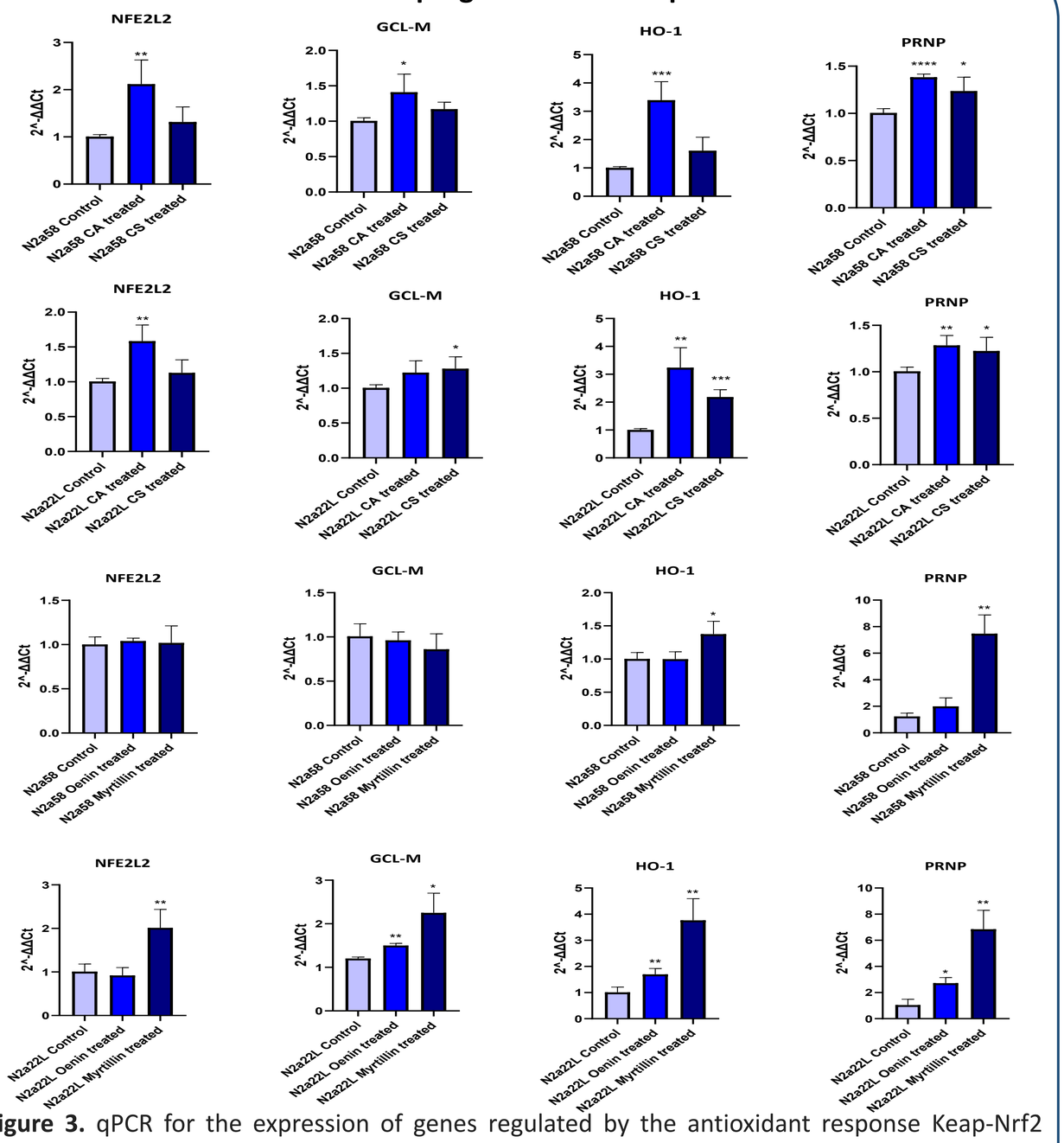


**Figure 3.** qPCR for the expression of genes regulated by the antioxidant response Keap-Nrf2 pathway and *PRNP* was performed using mRNA extracted from N2a58 and N2a22L cells treated with 0.5 × $LD_{50}$ of Anthocyanins for 48 h and compared to non-treated control cells (Cntrl).

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.

### Anthocyanins inhibit the *De Novo* Formation of the conversion of $PrP^{Sc}$ aggregates

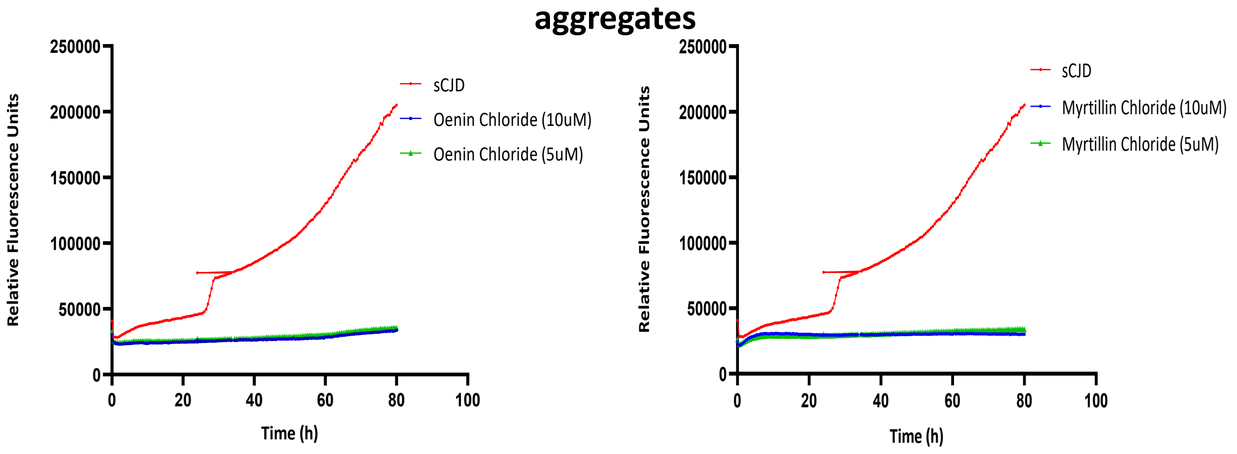


**Figure 4.** RT-QuIC assays. Aggregation of $recPrP^{C}$ in RT-QuIC assays using CSF from three different sCJD patients as a seed was evaluated in the presence or absence of Oenin Chloride and Myrtillin Chloride (5 and 10 mM final concentration). Oenin Chloride and Myrtillin Chloride were added to the reactions from the beginning, and the Th-T fluorescence was recorded every 30 min.

## 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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