ASSESSMENT OF NEUROPROCTECTION EFFECTS OF THE DIETARY SUPPLEMENT "ADN TÉLOMÉRACTIVES" ON ALZHEIMER AND PARKINSON MODELS

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1 - AIM OF THE STUDY

The aim of this study was to assess the *in vitro* neuroprotection effects the dietary supplement "ADN Téloméractives" toward Alzheimer's cell models used to study neurodegenerative diseases. Neurodegenerative diseases models can be set up in laboratory using differentiated neuron cell lines. With chemicals inducers, Alzheimer's diseases models are obtained from the human neuroblastoma SH-SY5Y cell line. The neuroprotective properties of the two candidates are assessed following a neurotoxicity assay, by counting neurons and measuring neurites length.

2 - MATERIALS

2.1 Cell culture

Table 1: Cell culture materials

Material	Supplier	Reference	Batch
SH-SY5Y	ATCC	CRL-2266	13C014
Trypsin / EDTA	Promocell	C41000	417M214
HAM F12	EUROBIO	CM1H120001	690434
Fetal Calf Serum	EUROBIO	S1810-500	-
Greiner advanced 96 wells plates	Dutscher	655986	E160433M

2.2 Chemicals

Table 2: Chemicals

Compounds	Supplier	Reference
Aftin	ManRos	-
AND Téloméractives comprimés	HBN	Lot 002 07/2018

2.3 Immunostaining

Table 3: Immunostaining materials

Materials	Supplier	Reference
PBS 1X	Eurobio	CS1PBS01
Acetone	Sigma	24201-25-R
Methanol	Sigma	32213-25L
Hoecsht 33258	Sigma	B2883

Rabbit polyclonal antibody to Tubulin 3	Abcam	Ab18207
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Molecular Probes	A11034

3 - METHODS

3.1 Cell culture and differentiation

SH-SY5Y were grown and subcultured according to supplier's recommendations.

All assays have been done in technical triplicate using 96 wells microplates. SH-SY5Y cells were differentiated by exposure to retinoic acid (10µM) for 4 days.

3.2 Chemicals

The extract was prepared following our customer recommendation. The working solutions were freshly prepared with a 12 final concentrations range from 0.00125 – 0.0025 – 0.0050 – 0.0125 – 0.025 – 0.05 -0.125 – 0.25 – 0.5 – 1.25 mg/mL.

3.3 Cell fixation and staining

The cells were then fixed using a mix of Ethanol and acetone at room temperature for 5 minutes, prior to immunostaining of nuclei with Hoechst and B3 tubulin with a primary antibody anti-B3 tubulin and a secondary antibody conjugated to an Alexa 488 fluorochrome.

3.4 Neuroprotection assay

Cytotoxicity was assessed using cell nucleus staining and cell counting.

The neuroprotective properties of the two candidates were assessed following a neurotoxicity assay based on neurons counting and neurites morphological assessment. Then, cells were exposed to aqueous extracts of the dietary supplement "ADN Téloméractives", with Aftin (Alzheimer's disease phenotype inducer) for 24 hours. After fixation and labelling, neurites outgrowth and cell count were assessed using MetaXpress (Molecular Devices).

3.5 Cell Imaging and images analysis

The images were acquired on an automated ImageXpress® Micro XLS microscope (Molecular Devices), with a 20X objective. 2 dichroic filters were used simultaneously to select specifically the detection of the different probes used. 4 fields per well have been acquired and analyzed individually.

The images were analyzed using MetaXpress (Molecular Devices) software.

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3.6 Statistical Analysis

Statistical comparison of results versus control conditions were performed using a ANOVA followed by Dunett test post hoc. Significance was expressed with the symbol " * " when $p<0.05$.

4 - RESULTS & DISCUSSION

4.1 Cytotoxicity

Cytotoxicity was assessed on differentiated SH-SY5Y after 24 hours of exposure. Product was tested co incubate with fixed concentrations of Aftin.

The aqueous extract of "ADN Téloméractive" in combination with aftin is cytotoxic at concentrations above 0.05 mg/mL. IC50 was evaluated at 0.022 mg/mL.
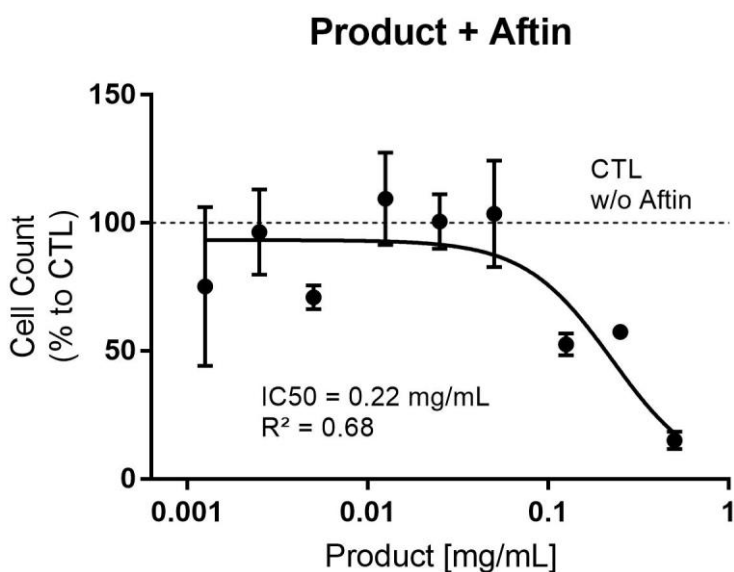

Figure 1: Cytotoxicity of the aqueous extract or "ADN Téloméractives", after 24 hours of exposure. Product was tested alone or co incubated with fixed concentrations of Aftin. 100% = cell count of the cells receiving medium alone. Results are expressed as a mean +/- SD of 3 replicates.

The highest concentration of aqueous extract tested (1.25 mg/mL) was not presented for IC50 calculation due to high toxicity.

4.2 Neuroprotective effect of product on neurites lengths on Alzheimer's model

The aqueous extract at 0.0025 and 0.0125 mg/mL significantly protected the neurites length against induction of Alzheimer phenotype after 24 hours of exposure to Aftin at 30 µM.
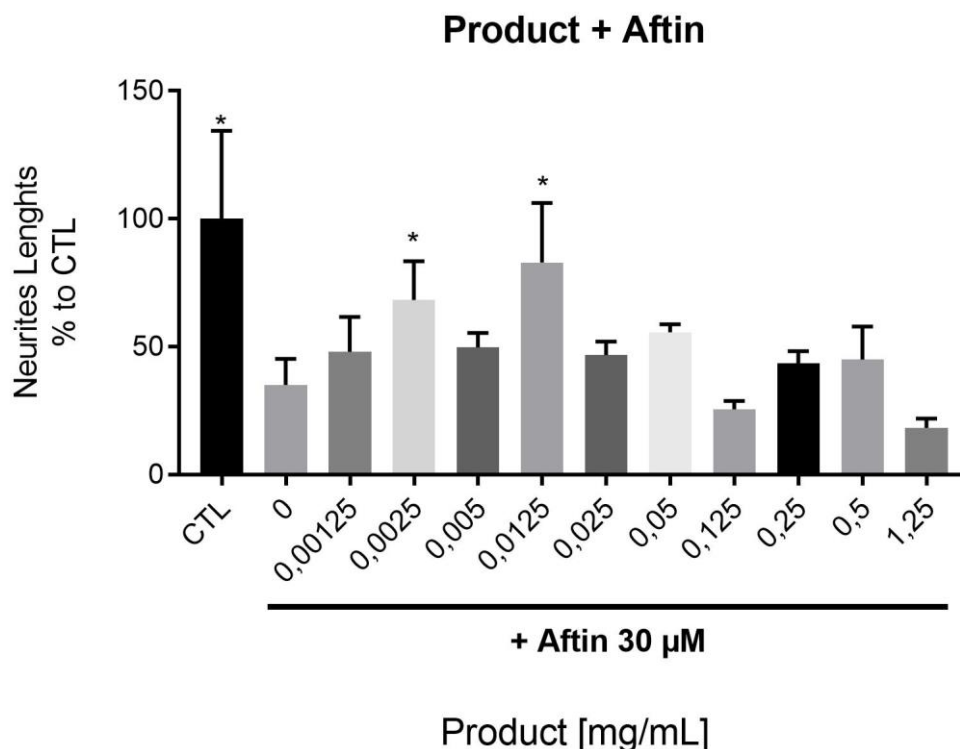

Figure 4: Neuroprotective effect of product on neurites lengths after 24 hours in Alzheimer model. Results are expressed as a mean +/- SD of 3 replicates.
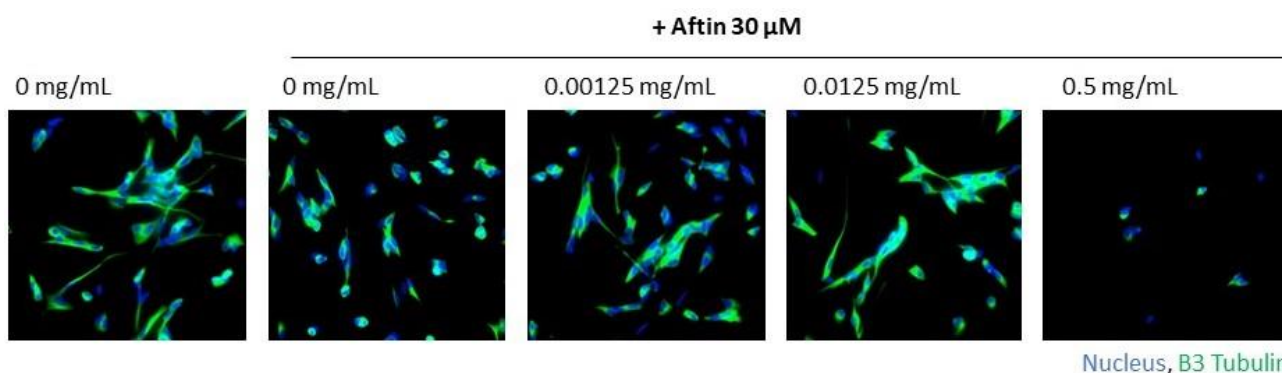

Figure 5: Illustrations of product dose effect on differentiated SH-SY5Y exposed to aftin 5. 20X magnification, nuclei in blue, B3 tubulin in green.

Differentiated SH-SY5Y cells when exposed to Aftin 5 generate an Alzheimer phenotype through the expression and secretion of Amyloid β42 protein. This amyloid protein is linked to the formation of amyloids plate, which characterize Alzheimer pathology. The aqueous extract at 0.0025 and 0.0125 mg/mL significantly protected the differentiated SH-SY5Y cells versus the deleterious effects of Aftin 5.

5 - DATA

Raw data are stored in excel files and can be provided on demand, or as an attached annex to the final report. Images in high quality can also be provided on demand through hard drive transfer.

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