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Data Article

Data on the effects of *Hyptis* spp. and *Lycium* spp. plant extracts in *C. elegans* models of genetically determined neurodegenerative diseases

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ABSTRACT

Here, we present the data on the biological effects of *Hyptis* spp. and *Lycium* spp. plant extracts in *Caenorhabditis elegans* (*C. elegans*) models of neurodegenerative diseases, which is related to the work presented in the article "Neurotherapeutic effect of *Hyptis* spp. leaf extracts in *C. elegans* models of tauopathy and polyglutamine disease: role of the glutathione

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redox cycle" [1]. This dataset was generated to define non-toxic concentrations of these plant extracts and to assess their impact on the motor phenotype and oxidative stress resistance of transgenic *C. elegans* models of two genetically defined neurodegenerative diseases: Machado-Joseph disease and Frontotemporal dementia with Parkinsonism associated to the chromosome 17. The impact of the plant extracts on toxicity was assessed using the food-clearance assay, absorbance being measured daily for seven days at 595 nm to quantify *Escherichia coli* (*E. coli*) strain OP50 bacteria consumption. Worm length and motor behaviour, including spontaneous and stimulated movement, were analysed using videos acquired with an Olympus SZX7 stereomicroscope with an integrated camera (Olympus SC30) and processed using the Image J® software and the Wrmtrck plugin. The resistance to oxidative stress induced by 240 µM juglone was assessed by determining the percentage of live animals after 1 hour of exposure.

Specifications Table

Subject	Molecular Medicine
Specific subject area	Drug discovery/chemical biology
Type of data	Table Image Figure
How data were acquired	Toxicity data were acquired through the daily measure of absorbance at 595 nm in the plate reader NanoQuant Plate™(Tecan) To evaluate the impact of plant extracts treatment on the motor phenotype of the *C. elegans* models of neurodegenerative diseases, videos of treated animals were acquired using an Olympus SZX7 stereomicroscope with an integrated camera (Olympus SC30) and processed using the Image J® software [2] and the Wrmtrck plugin [3]. Data on oxidative stress resistance were obtained by the scoring of survival of adult animals (in an Olympus SZX7 stereomicroscope), chronically treated with extracts and submitted to an oxidative insult caused by juglone.
Data format	Raw Analyzed
Parameters for data collection	Methanolic extracts of: leafs of *Hyptis suaveolens, Hyptis pectinata, Hyptis marrubioides*; and fruits of *Lycium barbarum* and *Lycium ruthenicum,* were used to chronically treat wild-type and *C. elegans* transgenic models of neurodegenerative diseases: Frontotemporal dementia with parkinsonism linked to chromosome 17 (human mutant tau V337M) [4] and Machado-Joseph disease (human mutant ATXN3 with 130 polyglutamines) [5], until day 4 after hatching. *C. elegans* models of neurodegenerative diseases, chronically treated with the plant extracts were also exposured to juglone for 1 hour.
Description of data collection	Toxicity data were estimated in wild-type treated animals through the estimation of bacteria (*E. coli* strain OP50) consumption, for 7 days, measured at 595 nm (NanoQuant Plate™-Tecan). The effect of the chronic treatment with plant extracts in disease models was evaluated through the estimation of animals' mean velocity. For this, populations of adult treated animals were recorded for 60 seconds while moving spontaneously and 30 seconds after being stimulated by a mechanic stimulus (plate tap). Oxidative stress resistance was evaluated submitting the *C. elegans* disease models, chronically treated with the plant extracts, to 240 µM of juglone for 1 hour. After 16 hours of recovery, dead/alive animals were counted.

(*continued on next page*)

Data source location	University of Minho, Braga Portugal
Data accessibility	With the article. The raw data is in the Mendeley Data repository, with the following link: http://dx.doi.org/10.17632/8v27k37dg2.1. The detailed data and statistical analysis are in supplementary data in appendix A.
Related research article	D. Vilasboas-Campos, M.D. Costa, A. Teixeira-Castro, R. Rios, F.G. Silva, C. Bessa, A.C.P. Dias, P. Maciel, Neurotherapeutic effect of Hyptis spp. leaf extracts in Caenorhabditis elegans models of tauopathy and polyglutamine disease: role of the glutathione redox cycle, Free Radic. Biol. Med. (2020). https://doi.org/10.1016/j.freeradbiomed.2020.10.018.

Value of the Data

- These data provide new information about the impact of distinct plant extracts on nematode models of neurodegenerative diseases, clarifying their potential use as sources of therapeutic compounds for such incurable disorders.
- Identification of bioactivities of natural compounds can be relevant for the discovery of new therapeutic agents to benefit patients with neurodegenerative diseases.
- This data can guide future research on these or other plant extracts or on their bioactive isolated compounds, towards the identification of new therapeutic agents for human diseases.

1. Data Description

The dataset of this study was used to investigate the toxicity of distinct plant extracts *in vivo* as well as their effects on motor function and oxidative stress resistance of *C. elegans* models of neurodegenerative diseases (Tauopathies and Machado-Joseph disease) as exemplified in the experimental workflow shown in Experimental design, Materials and Methods section.

1.1. Non-toxic concentrations

In order to define the concentrations of *Lycium ruthenicum* (LR) and *Lycium barbarum* (LB) plant extracts to be used in the following experiments, we first assessed the toxicity of different concentrations of these plant extracts in wild-type (WT) worms. For this, the profile of bacterial food consumption by *Lycium*-treated animals was measured daily and used as proxy of worm development and well-being (RAW data in Dataset R1 [6]). None of the *Lycium* plant extracts concentrations tested (0.25 to 1 mg/mL) led to differences in food consumption measured by OD595 nm when compared to the non-toxic control DMSO 1% (Fig. 1, Table S1 (Appendix A)). In the case of *Hyptis suaveolens* (HS), *Hyptis pectinata* (HP) and *Hyptis marrubioides* (HM) extracts, OD595 nm measures in treatment conditions were also similar to the non-toxic control [1].

1.2. Effect of plant extracts treatment on the speed of the C. elegans *model of tauopathy, during spontaneous and stimulated movement*

In a transgenic *C. elegans* model of the human tauopathy Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), that expresses pan-neuronally a full-length mutant Tau V337M [4], the chronic treatment with HS, HP and HM plant extracts increased significantly the stimulated speed of worms, at the highest concentrations, when compared with

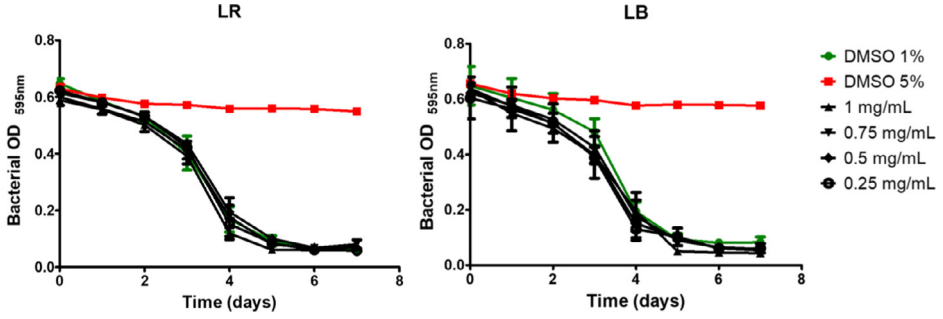

Fig. 1. Consumption of bacterial food by WT *C. elegans* treated with distinct concentrations of *Lycium ruthenicum (LR)* and *Lycium barbarum* (LB) (0.25-1 mg/mL). Animals were also treated with DMSO 1% (non-toxic treatment) and DMSO 5% (toxic treatment). Bacterial consumption was estimated through optical density (OD) measurement at 595 nm for 7 days. Graphs are representative of one independent assessment with at least three technical replicates being considered for each timepoint and condition.

the DMSO treatment: at 0.5 and 1 mg/mL for HS and HP, and at 0.1 and 0.5 mg/mL for HM (Fig. 2, Table S2 (Appendix A) and Dataset R2 [6]). However, the treatment of the same model with LB and LR plant extracts did not change velocity in comparison with the control condition. (Fig. 2, Table S2 (Appendix A) and Dataset R2 [6]).

Similarly, when the animals' speed was evaluated during their spontaneous movement, velocity was higher for HS, HP and HM treatment [1], and no major effect in speed was seen upon treatment with LB and LR plant extracts (Fig. 3, Table S3 (Appendix A) and Dataset R2 [6]). Of note, treatment with the distinct extracts did not alter the overall development/size of the transgenic *C. elegans* model of Tauopathy (Table S2 and S3 (Appendix A), Dataset R3 [6]).

1.3. Effect of plant extracts treatment on the speed of the C. elegans *model of MJD, during spontaneous and stimulated movement*

The effect of plant extracts treatment was also tested in a *C. elegans* model of Machado-Joseph disease (MJD), previously generated and characterized in our laboratory, which expresses pan-neuronally a mutant version of human ATXN3 (mATXN3) and exhibits locomotion defects as a key hallmark of the disease [5].

In that case, none of the plant extracts was able to improve animals speed, when the movement was stimulated by tapping the Petri dish (Fig. 4, Table S4 (Appendix A) and Dataset R4 [6]). When evaluated in spontaneous movement, *Hyptis*-treated worms presented significantly increased velocity, when compared with the DMSO control condition [1], however, LB and LR treatments were unable to change animals' velocity (Fig. 5, Table S5 (Appendix A) and Dataset R4 [6]).

Also, in this case, the body length of the *C. elegans* model of MJD, treated with the distinct extracts, was comparable to the DMSO treatment (Table S4 and S5 (Appendix A), Dataset R5 [6]).

1.4. Oxidative stress resistance of the C. elegans *models of neurodegenerative diseases treated with* Hyptis *spp. and* Lycium *spp. plant extracts*

The *C. elegans* models of neurodegenerative diseases, pre-treated with 1 mg/mL of each extract, were submitted to an oxidative insult triggered by juglone (240 µM). The treatment with *Hyptis* spp. extracts led to increased oxidative stress resistance in both disease models [1]. Concerning the treatment with LR and LB extracts, only the model of tauopathy presented increased

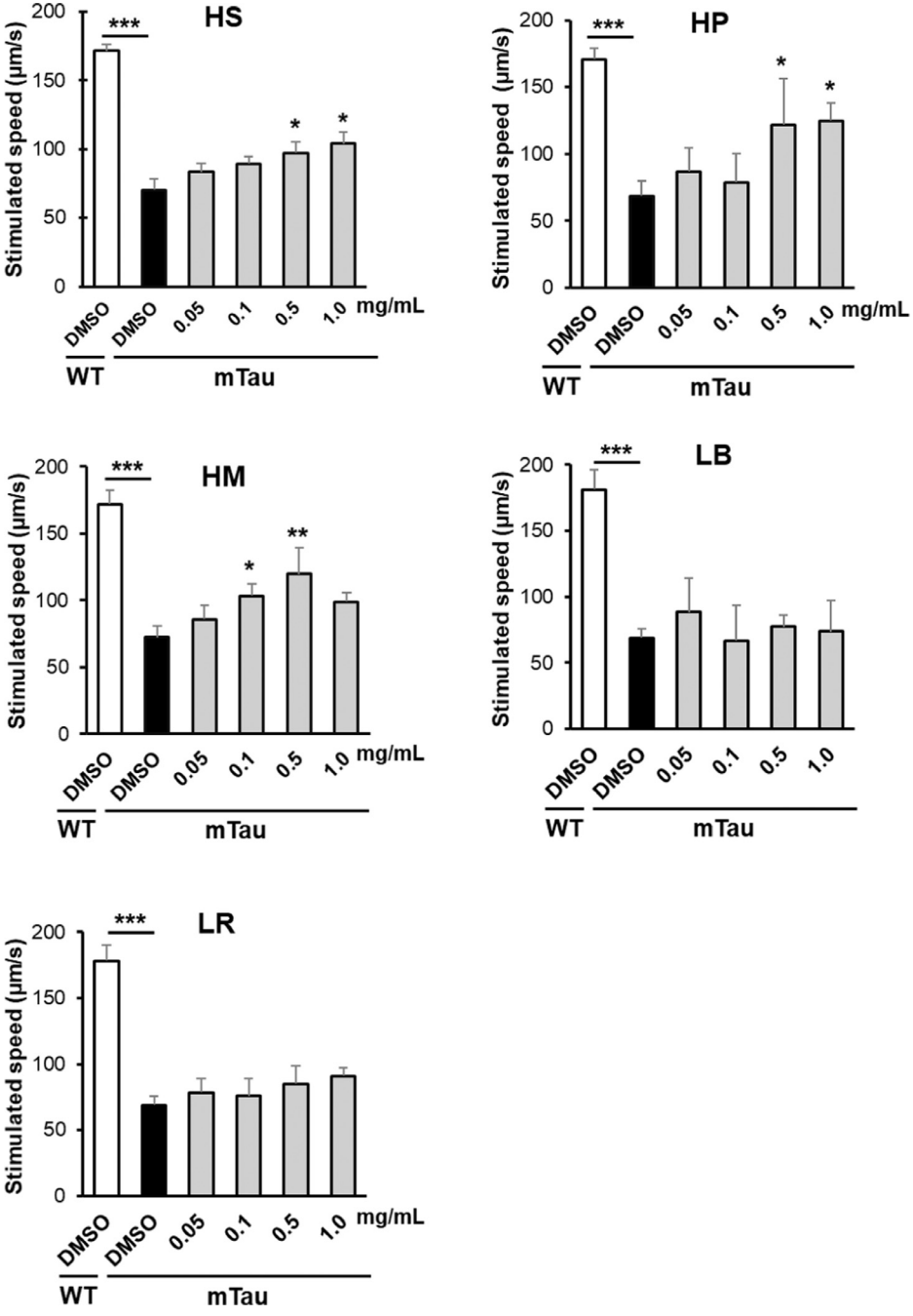

Fig. 2. Mean velocity of mTau animals treated with different concentrations of plant extracts, when their movement was stimulated by "tapping". Each bar represents means ± SEM of 3-9 experiments with at least 50 worms analysed in each assay per condition. $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***), One-way ANOVA followed by the bilateral Dunnet test (post hoc).

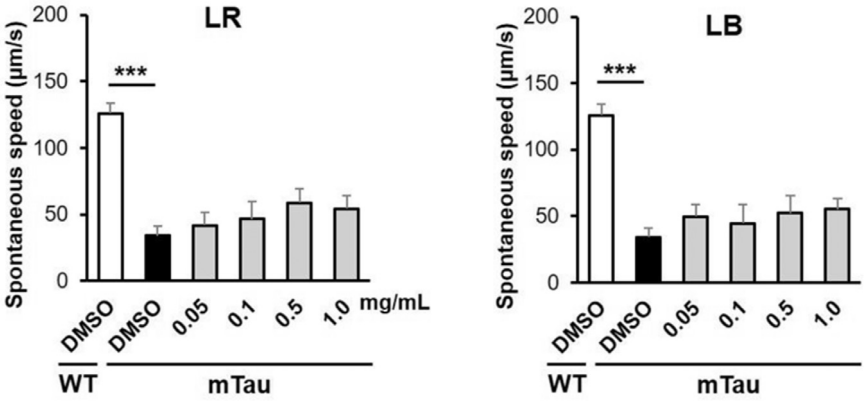

Fig. 3. Mean spontaneous speed of mTau animals treated with different concentrations of LB and LR plant extracts. Each bar represents means ± SEM of 9 experiments with at least 50 worms analysed in each assay per condition. $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***), One-way ANOVA followed by the bilateral Dunnet test (post hoc).

survival percentage upon juglone exposure when chronically treated with the LR extract (Fig. 6, Table S6 (Appendix A), Datasets R6 and R7 [6]).

2. Experimental Design, Materials and Methods

2.1. Plant extracts

Leaves of *Hyptis suaveolens, Hyptis pectinata* and *Hyptis marrubioides* plants were collected in the region of Rio Verde (Goiás, Brazil) and fruits of *Lycium barbarum* and *Lycium ruthenicum* were collected in China. Extracts were prepared as described in [1]. Briefly, biomass was lyophilized and extracted with methanol 80%, in the dark, for 5 days. After this, solutions were filtered, evaporated in a rotavapor (in the dark, at 40 °C), lyophilized to obtain a powder and stored at -20°C. For the *in vivo* tests, the plant extracts powder was dissolved in DMSO 100% and stored as a stock concentration of 100 mg/mL at 4°C, protected from light.

2.2. C. elegans *strains, maintenance and synchronization*

The *C. elegans* model of MJD was previously generated by our research team [5], the *C. elegans* model of FTDP-17 was kindly provided by Dr. Brian Kraemer and wild-type strain (N2; Bristol strain) was obtained from CGC.

Animals were grown at 20°C on nematode growth medium (NGM) agar plates seeded with *E. coli* strain OP50. The synchronization of *C. elegans* was achieved by hypochlorite treatment (~2.6% NaClO and 0.5 M NaOH) for 5 min.

2.3. Toxicity assessment

The extracts toxicity was evaluated in the *C. elegans* wild-type Bristol strain N2 as exemplified in the experimental design in Fig. 7, in Section 2. A food-clearance assay was performed in liquid culture using 96-well plates, as already described [7]. Each well contained a final volume

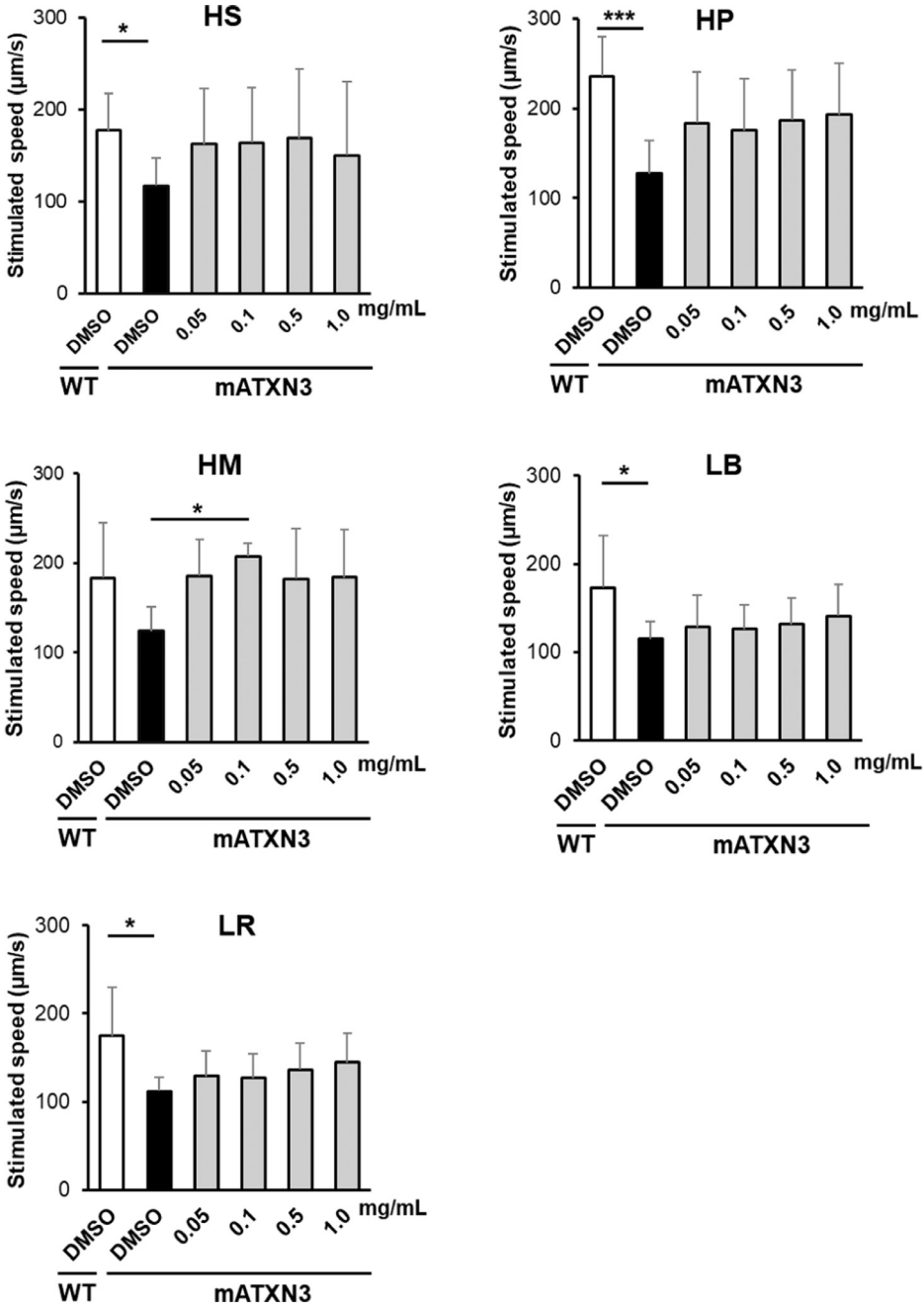

Fig. 4. Mean stimulated velocity of mATXN3 animals treated with different concentrations of plant extracts. Each bar represents means ± SEM of 3-9 experiments with at least 50 worms analysed in each assay per condition. $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***), One-way ANOVA followed by the bilateral Dunnet test (post hoc).

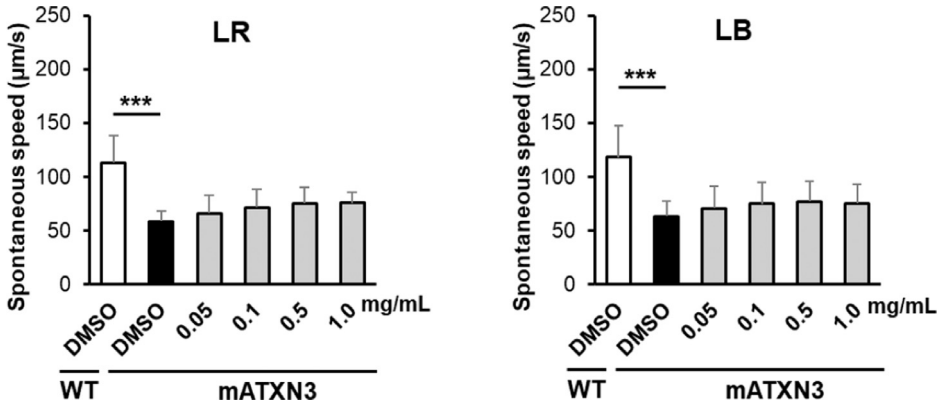

Fig. 5. Mean spontaneous velocity of mATXN3 animals treated with different concentrations of plant extracts. Each bar represents means ± SEM of 9 experiments with at least 50 worms analysed in each assay per condition. $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***), One-way ANOVA followed by the bilateral Dunnet test (post hoc).

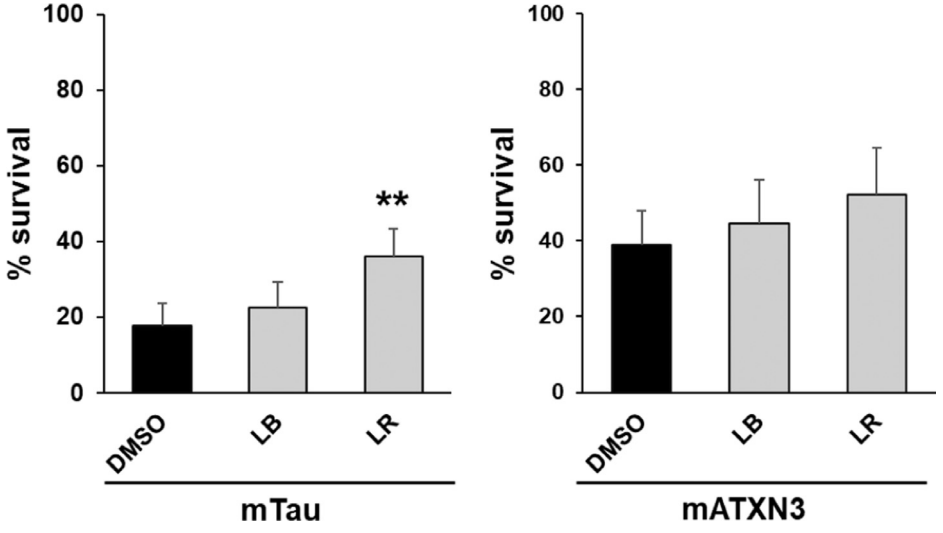

Fig. 6. Effect of *Lycium* spp. extracts treatment (at 1mg/mL) on the oxidative stress resistance of the *C. elegans* models of MJD and Tauopathy. Oxidative stress was induced by exposure to 240 µM of juglone, for 1 hour. Data on the graph are represented as mean survival percentages ± SEM from 5 independent experiments. (N=~1000 animals). $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***), One-way ANOVA following bilateral Dunnet correction.

of 60 µL, comprising 15 µL of eggs suspension (20–25 eggs), 20 µL of *E. coli* strain OP50 bacteria (previously inactivated by cycles of freeze/thawing, with OD 595 nm of 0.6–0.7) and 25 µL of each plant extract (at different concentrations) or DMSO 1% and 5% (non-toxic and toxic treatments, respectively). Worms were grown with continuous shaking at 180 rpm at 20°C (Shell Lab incubator shaker). The consumption of the *E. coli* strain OP50 was measured daily for 7 days at 595 nm (NanoQuant Plate™-Tecan).

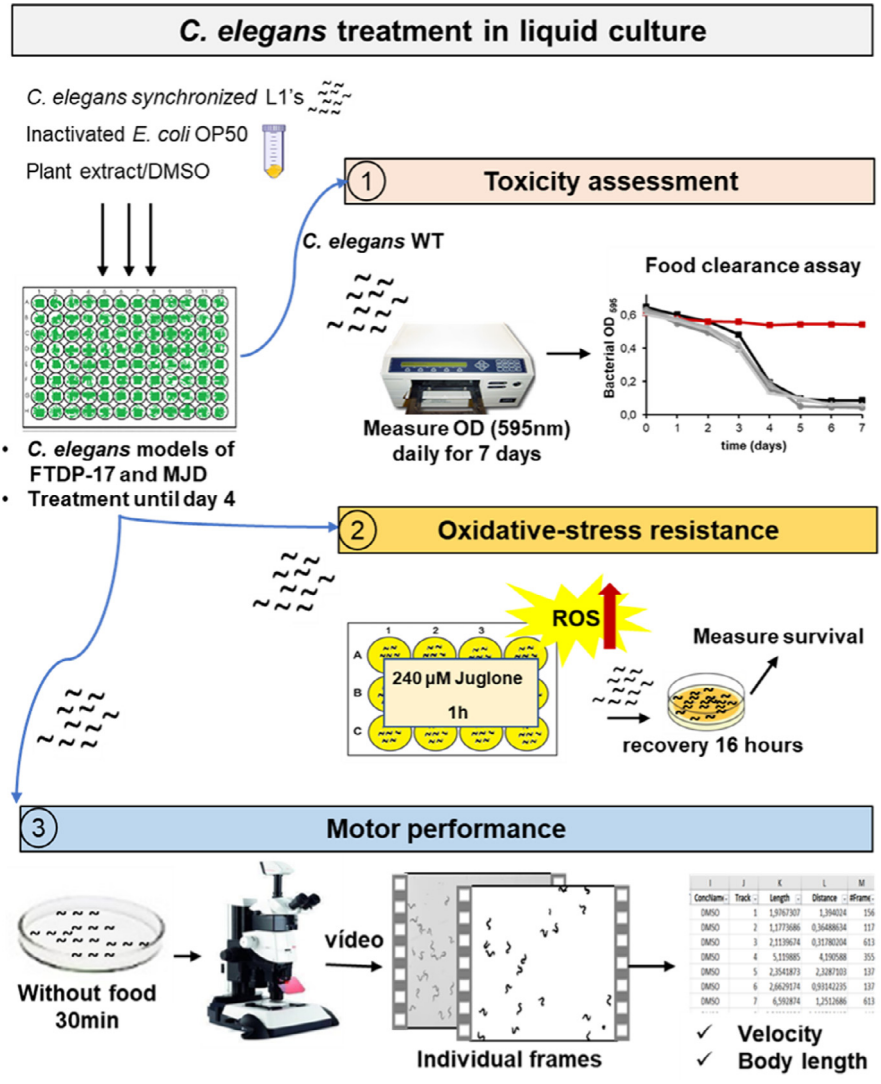

Fig. 7. Schematic representation of the experimental workflow used to evaluate the impact of plant extracts treatment in *C. elegans* models of neurodegenerative diseases: 1) toxicity of the plant extracts in WT strain 2) oxidative stress resistance (after juglone insult) and 3) evaluation of motor function (animals' spontaneous/stimulated speed), in the chronically treated *C. elegans* models of neurodegenerative diseases.

2.4. Motor phenotype

2.4.1. Chronic treatment of the C. elegans with plant extracts

The chronic treatment of *C. elegans* with plant extracts (from L1 to day 4 after hatching) was performed in liquid culture using 96-well-plates. The treatment plates comprised per well: 45-50 L1 animals; plant extracts at the respective concentrations and DMSO 1% as control; and *E. coli* strain OP50 inactivated bacteria resuspended in S-medium complete to a final OD 595 nm of 0.8-0.9. The plates were incubated at 20°C with shaking at 180 rpm (Shell lab incubator shaker).

2.4.2. Motor behavior assays in the C. elegans *disease models*

The motor phenotype of the MJD and Tauopathy *C. elegans* models was evaluated after treatment with the plant extracts as exemplified in the experimental design in Fig. 7, in Section 2. At day 4 after hatching, the animals were removed from the liquid medium to unseeded NGM plates, for 30 minutes. A population of animals in the NGM plate was recorded using an Olympus SZX7 stereomicroscope with an integrated camera (Olympus SC30), for 1 minute while moving spontaneously (in each trial and for each condition two videos were recorded) or for 30 seconds while moving after stimulated by a mechanic stimulus (plate tap). To ensure that the movement stimulus was similar throughout the procedure, the plates were dropped from a height of 8 cm, three consecutive times, before being placed in the stage plate. In that case, in each trial and for each condition one video was recorded. The videos were processed using the Image J® software [2] and the Wrmtrck plugin [3].

2.5. Resistance to juglone-induced oxidative stress

Transgenic *C. elegans* models of MJD and FTDP-17, chronically treated with plant extracts until day 4 after hatching, were washed three times in M9 buffer before incubation with a ROS generator, 5-Hydroxy-1,4-naphthoquinone (Sigma-Aldrich, St. Louis, MO), also known as juglone. Approximately 250-300 animals were incubated with 240 µM Juglone, for 1 hour at 20°C, in liquid medium (12-well plate), with continuous agitation as exemplified in the experimental design in Fig. 7, in Section 2. After oxidative stress, animals were washed with M9 buffer 5 times and moved to fresh NGM plates seeded with *E. coli* strain OP50 for a recovery period of 16 hours, when the survival percentage was determined by touch-provoked movement. Animals were scored as dead when they failed to respond after touching with a platinum wire pick.

2.6. Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics software (version 23.0). Continuous variables were tested for normal distribution (Shapiro–Wilk or Kolmogorov–Smirnov normality test), for homogeneity of variance (Levene's test) and outliers. Data were analyzed using One-Way Anova, Dunnett's multiple comparison analysis for post hoc comparison. All experiments were run at least in triplicate ($n \geq 3$) and data presented correspond to mean ± SEM with significance levels of $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***), respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2020.106598.

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