



NEUROSYS

CNS/PNS SERVICES

From traditional use to standardized and optimized neuroprotective extract of *Huperzia serrata*: NSP01

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INTRODUCTION

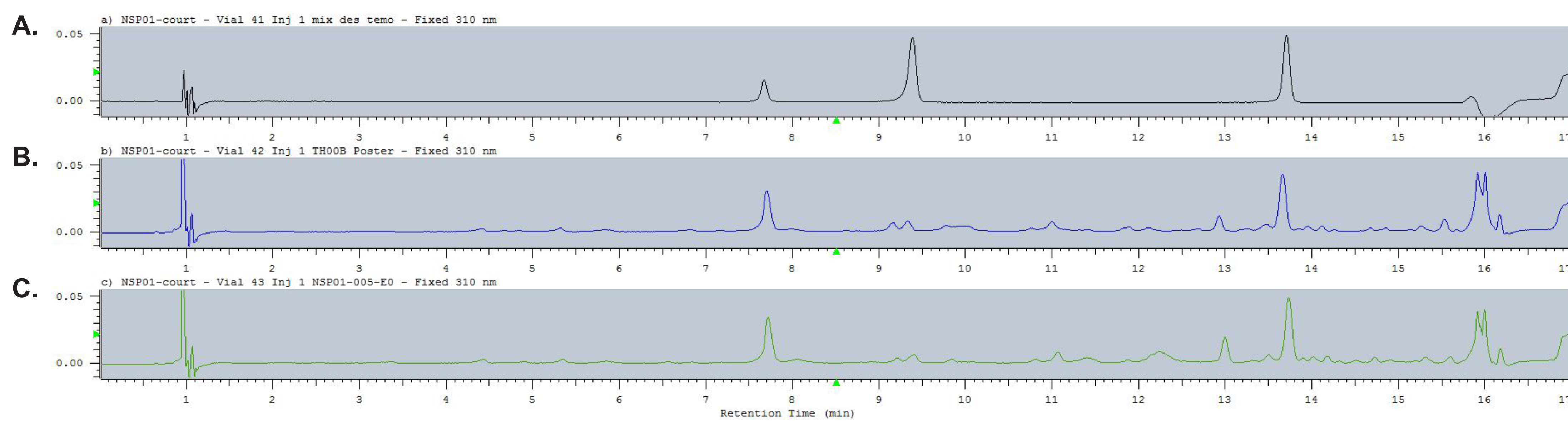
Alzheimer's disease (AD) is the principal type of dementia in adults and represents about 70 % of patients suffering from dementia. AD is a chronic neurodegenerative disease that manifests as progressive memory loss and cognitive decline. The neuropathologic hallmarks of AD are senile plaques, presence of neurofibrillary tangles and dystrophic neurites in neurons. The amyloid and tangle cascade hypothesis is the currently admitted hypothesis explaining the pathogenesis of AD (Jan *et al.* 2010, Callizot *et al.*, 2013).

Huperzia serrata (HS) is used in Asiatic folk medicine, via infusion, for their neuromuscular and memory-improving properties. Huperzine A (HA) is one of the constituents of *H. serrata* and was proven to be a reversible inhibitor of acetylcholinesterase (AChE). The results on more than 100.000 Chinese patients indicated that HA significantly improved cognitive functions, then could be a serious alternative treatment for Alzheimer's disease (AD). However, clinical trials of HA treatment also indicated that the efficient doses in AD patients were the ones displaying some advent events (headaches, digestive or cardiac disorders...).

We have developed an optimized "green extract" called NSP01 (using an eco-extraction process) of HS. The neuroprotective effect on *in vitro* models of AD was compared to the one of the "traditional" extract. We showed that NSP01 displayed a significant neuroprotective effect comparable and even most efficient than the traditional extract. We proved that the neuroprotective effect of NSP01 was based on the synergistic activity between 3 molecules: HA and phenolic acids (Caffeic and Ferulic acids). This synergy was based on a precise ratio between the molecules and allows a neuroprotective effect at doses in that each molecule used alone was inactive and then at doses of HA without any cholinergic adverse side effects. NSP01 is patented and commercialized as a food supplement for neuro-indication such as cognitive impairment.

RESULTS

Comparative composition of NSP01 and TE



Neuralia

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METHODS

Plant material: The plant material (whole plant) of *H. serrata* was collected in Bi Doup, Vietnam, sorted, washed with water and brushed to remove impurities. To remove microbial contaminations, the plant was treated with a chloramine B solution (5 %) during 15 min. Then, the plant was chopped into small pieces and dried for 12 h, at 40-45 °C, until desiccated. The plant was identified and confirmed by Prof. Tran Hop (Department of Botany, Ho Chi Minh City, Vietnam).

Extraction: Traditional extract (TE) was prepared by decoction for 30 minutes in a boiling water bath. After the crude extract was filtered through cotton sheet. The filtered solution was frozen at -20 °C and freeze-dried to produce dried powder. The microwave-assisted extract (NSP01) was prepared by extraction of air-dried plant material with demineralized water for 30 minutes at 200 W using microwave assisted extractor Milestone Ethos EX (Milestone, Italy).

UHPLC-DAD-ESI-MS/MS analysis was performed on Thermo Ultimate 3000 Ultra High Performance Liquid chromatograph with quaternary pump system, connected to a DAD detector. Chromatographic separation was achieved using Thermo Acclaim RSLC 120 C18 column (150x2.1 mm, 2.2 µm, Thermo Fisher Scientific, USA) column, operated at 45 °C.

Pharmacological evaluation: Culture of cortical neurons: Primary rat cortical neurons (E15) were cultured as described by Callizot *et al.*, 2013. The cortical cells were seeded at a density of 25,000 per well in poly-L-lysine pre-coated 96-well plates and cultured at 37 °C in an air (95 %)-CO₂ (5 %) incubator. On day 13 of culture, TE, NSP01, molecules or mixture of compounds were pre-incubated for 1 hour before Glutamate or Amyloid 1-42 peptide exposure. 48 or 24 h after injury in presence of the compounds, the cultures were analyzed.

Robotized platform (HCS system): End point evaluation: Neuron survival: 24h or 48h after intoxication, cortical neurons were stained with anti-MAP-2 anti-body. Analysis of total number of neurons was performed automatically (30 randomly selected pictures per well; 20 x magnification). All data were expressed in percentage of control conditions (no intoxication = 100 %). All values expressed as mean +/- SEM (standard error of the mean) (n = 6 wells per condition per culture). ANOVA followed by the LSD Fisher's test were performed.



Huperzia serrata

Figure 1: Analytical profile of TE and NSP01

Chromatograms (UHPLC-ELSD) of:

- (A) reference mix: Huperzine A (HA), Caffeic acid (CA) and Ferulic acid (FA)
- (B) traditional extract (TE)
- (C) Optimized and NSP01

Neuroprotective effect of NSP01 after glutamate (A and B) or Amyloid peptide injuries (C)

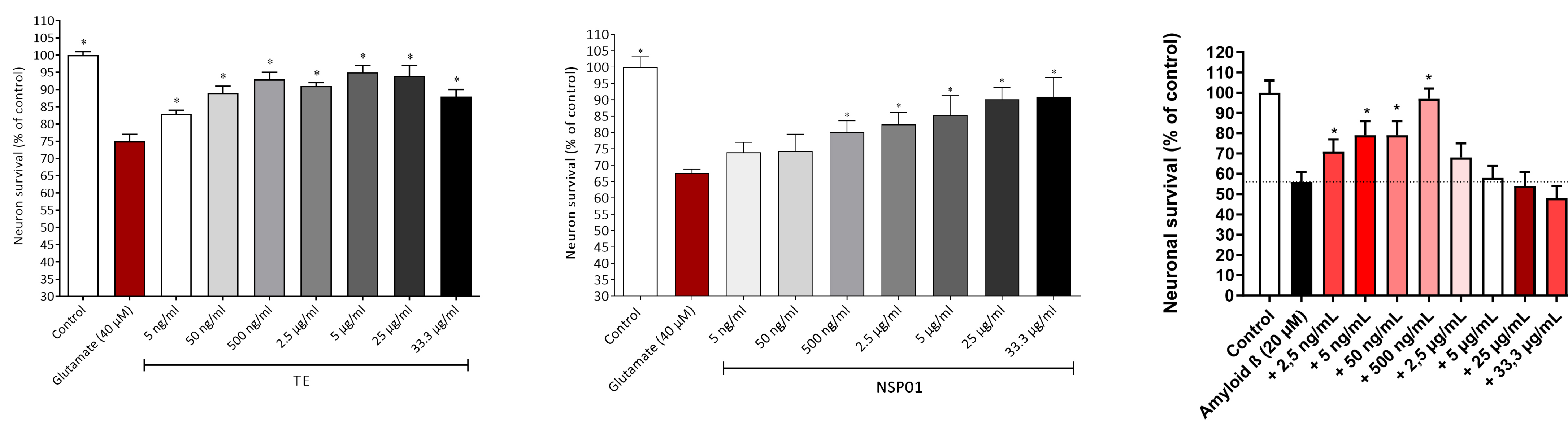


Figure 2: Neuroprotective effect of NSP01 and TE after a neuronal injury

NSP01 (at different doses) and TE were able to protect primary cortical neurons from glutamate toxicity.

NSP01 was able to protect neurons injured wby the Amyloid peptide following a bell shape curve effect.

* p<0,05 vs control. One-way ANOVA followed by Dunnett's test

Synergistic effect of Huperzine A, caffeic acid and ferulic acid: the 3 major components of the NSP01

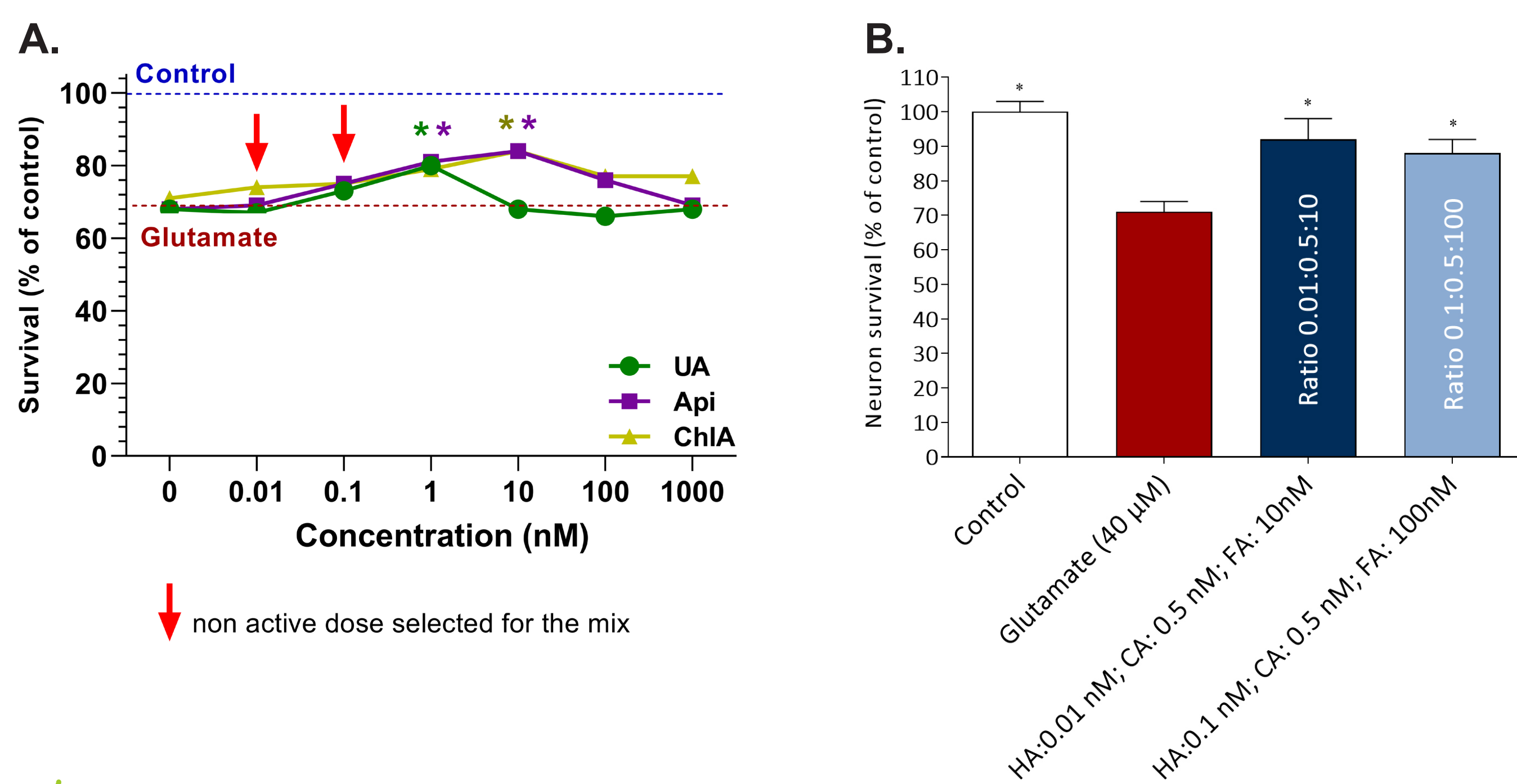


Figure 3: Synergistic effect of the 3 main components of NSP01

Neuronal survival of cortical neurons injured with glutamate in the presence or not of HA, CA and FA (figure A)

Neuronal survival after glutamate injuries of primary cortical neurons with or without the 3 molecules mixed at a specific ratio (figure B).

* p<0,05 vs control. One-way ANOVA followed by Dunnett's test



CONCLUSIONS

- NSP01 ("green and optimized extract" of *H. serrata*) showed a neuroprotective effect in *in vitro* models of AD;
- NSP01 was enriched in 3 main molecules: Huperzine A, caffeic and ferulic acids, that support the neuro-activity of NSP01 under a specific ratio of each molecule;
- Synergistic neuroprotective activity of NSP01 should allow to decrease the HA cholinergic side effects as the dose of HA in the NSP01 was decreased.

NSP01 developed by Neuralia® is a very promising optimized plant extract for memory deficits. (For more information: www.neuralia-nutraceuticals.com)