From traditional use to standardized neuroprotective green-extract of Huperzia serrata

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INTRODUCTION

Some plants belonging to the Lycopodiaceae family such as Huperzia serrata (Thunb.) Trevis (Fig. 1), A. squamosa (Forest.) Trevis, Lycopodium complanatum L. and L. cernus (L.) Franco & Vasc have been used in Asian folk medicine for thousands of years to treat contusions, strains, schizophrenia and memory dysfunction [1, 2]. H. serrata and H. saururus are used respectively in Chinese and Argentinian folk medicine, via infusion, for their neuromuscular and memory-improving properties [3, 4]. Huperzine A, one of the constituents of H. serrata, was proven to be a reversible inhibitor of acetylcholinesterase with neuroprotective effects [5]. The results on more than 100,000 Chinese patients indicated that Huperzine A significantly improved cognitive functions [6] and had the potential to become an alternative treatment for Alzheimer’s disease (AD). However, clinical trials with Huperzine A treatment also indicated that large doses of this compound and had the potential to become an alternative treatment for Alzheimer’s disease (AD).

METHODS

Plant material: whole plant of wild Huperzia serrata was harvested in Vietnam in 2013. The plant was certified by Pr. Tran Hinh and voucher specimen (TH000) was deposited in the laboratory of Neuro-Sys.

Extraction: traditional extract (TE) was prepared by boiling 10 g of ground plant in 150 mL of water for 30 minutes. After filtration through cotton the filtrate was freeze dried. A microwave extractor Ethos X (Milestone, Italy) was used in the preparation of NSP01 (batch 000-E0003a).

Chromatographic analysis was realized using Hitachi Chromaster Ultra RS system coupled to DAD detector. 0.1 % of formic acid in water and 0.1 % of formic acid in methanol were used as mobile phase. The separation was achieved on the Thermo-Scientific RSLC 120 C18 column (150 × 2.1 mm, 2.2 µm).

Pharmacological evaluation: Culture of cortical neurons: Primary rat cortical neurons were cultured as described in [7]. After 6 days of culture, cells were transfected and resuspended in a defined culture medium. The cells were seeded at a density of 25,000 cells per well in 96-well pre-coated 96-well plates and cultured at 37 °C in an air (95 %)-CO2 (5 %) incubator. The medium was changed every 2 days. On day 13 of culture, extract or compound and/or mix of compounds were solved in culture medium and pre-incubated for 1 h hour before glutamate stimulation. The cells were intoxicated with glutamate solution (40 µmol/L) for 20 min. After 20 min exposure, glutamate was washed out and fresh culture medium with extract or compound and/or mix of compounds was added for additional 48 h hours. Robotized platform (HCS system): End point evaluation: Neuron survival: 48 hours after intoxication, cortical neurons were stained with anti-MAP-2 antibody and revealed with Alexa Fluor IgG. 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, no glutamate = 100%). All values expressed as mean ± SEM (standard error of the mean) (n = 6 wells per condition per culture). ANOVA followed by the Fisher’s test when allowed.

RESULTS

A “Green” extract NSP01, obtained via microwave-assisted extraction, was compared to the TE. The superposition of chromatograms (Figure 2) showed comparable profiles for the two extracts. The content of Huperzine A was 0.21 % (m/m) in NSP01 vs 0.20 % (m/m) in the TE.

PHARMACOLOGICAL ACTIVITY OF TE AND NSP01 EXTRACTS

Effect of TE (1 h pre-treatment) on neuron survival after glutamate injuries (40 µmol/L, 20 min).

DISCOVERY OF SYNERGISTIC EFFECT OF THREE COMPOUNDS IDENTIFIED IN TE AND NSP01 EXTRACTS

Effect of mix of compounds (HA+CA+FA, 1 h pre-treatment) on neuron survival after glutamate injuries (40 µmol/L, 20 min).

CONCLUSIONS

These results showed the similarity of chromatographical profile and of the content of active compound (HA) of NSP01 vs. TE. The pharmacological activity of NSP01 lead to the discovery of synergistic activity between three compounds: Huperzine A, Caffeic acid and Ferulic acid. The active dose of Huperzine A was decreased from 1 nmol/L for a single compound to 10 pmol/L in a mix of three compounds. This synergistic activity should allow to decrease cholinergic side effects of high doses of Huperzine A.

Neuro-Sys is currently developing NSP01 as a food supplement for 2019.