Executive summary of the activities carried out during the implementation period

Project title: **Antiaggregation potential of 6-hydroxy-L-nicotine from Paenarthrobacter nicotinovorans pAO1 against amyloid peptide: in vitro and in vivo studies**

Contract No.: **PCE 49/2022**

Project code: **PN-III-P4-PCE-2021-1692**

Project manager: **Prof. univ. dr. habil. Lucian HRIȚCU**

Timeframe: **01/01/2023 – 31/12/2023**

In phase 2, all proposed activities were completed. The activities started with the quantification of the level of Aβ1-42 by the ELISA method and later with the cytotoxic activity (apoptosis) induced by Aβ1-42 by the DNA fragmentation method in hippocampal homogenates from NMRI mice and 5xFAD transgenic mice after exposure to 6HLN. We also assessed the impact of 6HLN on memory processes and the anxious and depressive responses of NMRI and 5xFAD transgenic animals. 6HLN was produced using a biotechnology-based on the microorganism *Paenarthrobacter nicotinovorans*.

The experimental results demonstrated that 6HLN (0.3 and 0.6 mg/kg) decreases the level of Aβ1-42 in fibrillar form in the brain of 5xFAD transgenic mice compared to the control groups (NMRI mice) (Fig. 1).

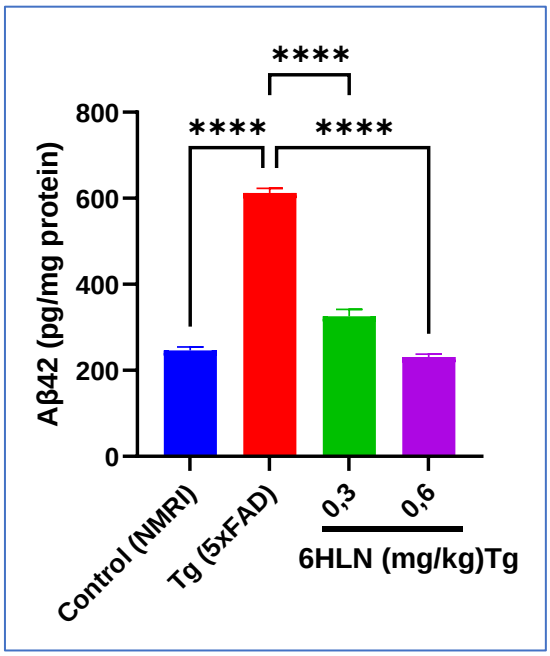

Fig. 1. Effects of 6-hydroxy-L-nicotine administration (6HLN, 0.3 and 0.6 mg/kg, g.c., i.p.) on the level of Aβ1-42 in the hippocampi of NMRI mice and 5xFAD transgenic mice. Results are expressed as means ± E.S.M. (n=5), using one-way ANOVA followed by Tukey's post hoc test ($p<0.0001$ for 0.3 mg/kg and 0.6 mg/kg).

Moreover, 6HLN (0.3 and 0.6 mg/kg) reduces Aβ1-42-induced cytotoxicity (apoptosis) by decreasing factor enrichment compared to control animals (NMRI) (Fig. 2).

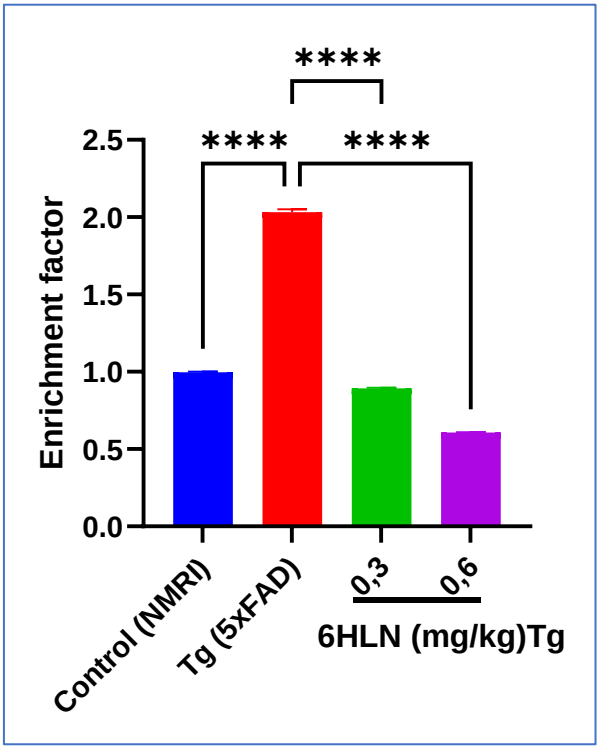

Fig. 2. Effects of 6-hydroxy-L-nicotine administration (6HLN, 0.3 and 0.6 mg/kg, g.c., i.p.) on apoptosis in the hippocampus of NMRI mice and 5xFAD transgenic mice evaluated by factor enrichment. Results are expressed as means ± E.S.M. (n=5), using one-way ANOVA followed by Tukey's post hoc test ($p<0.00001$ for 0.3 mg/kg and 0.6 mg/kg).

Administration of 6HLN (0.3 and 0.6 mg/kg) to 5xFAD animals attenuated deficits in spatial memory in the Y-maze test (Fig. 3) and working and reference memory in the radial arm-maze test (Fig. 4) compared to controls (5xFAD mice).

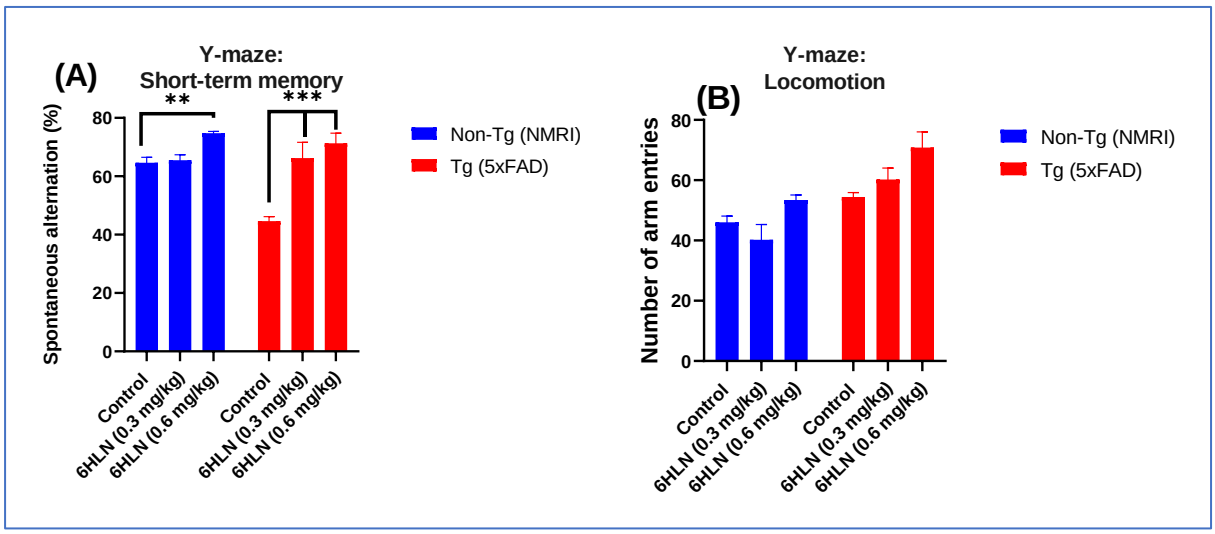

Fig. 3. Effects of 6-hydroxy-L-nicotine administration (6HLN, 0.3 and 0.6 mg/kg, g.c., i.p.) on short-term memory (A. Spontaneous alternation%) and locomotor activity (B. Number of entries in arms) in the Y-maze test. NMRI mice and transgenic Tg (5xFAD) mice were used. Results are expressed as means ± E.S.M. (n=5), using the two-way ANOVA test followed by Tukey's post hoc test (**$p<0.001$ and ***$p<0.0001$).

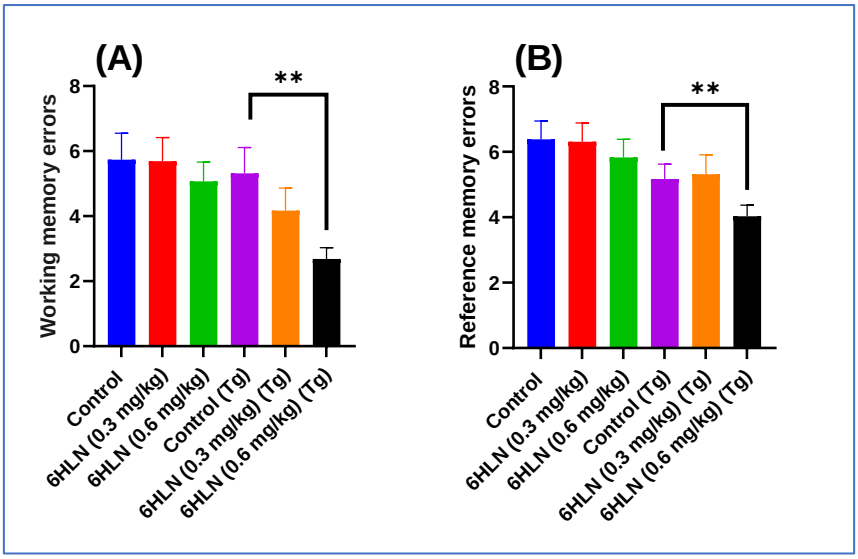

Fig. 4. Effects of 6-hydroxy-L-nicotine (6HLN, 0.3 and 0.6 mg/kg, g.c., i.p.) administration on working memory and reference memory performance in the radial arm-maze test. A. Working memory errors; B. Reference memory errors. NMRI mice and transgenic Tg (5xFAD) mice were used. Results are expressed as means ± E.S.M. (n=5), using the two-way ANOVA test followed by the post hoc Tukey test (**p<0.001).

Moreover, in 5xFAD mice pretreated with 6HLN (0.3 and 0.6 mg/kg) a reduction of the anxious response in the elevated plus maze test (Fig. 5) and the depressive response in the forced swimming test (Fig. 6) was found compared to the control groups (5xFAD).

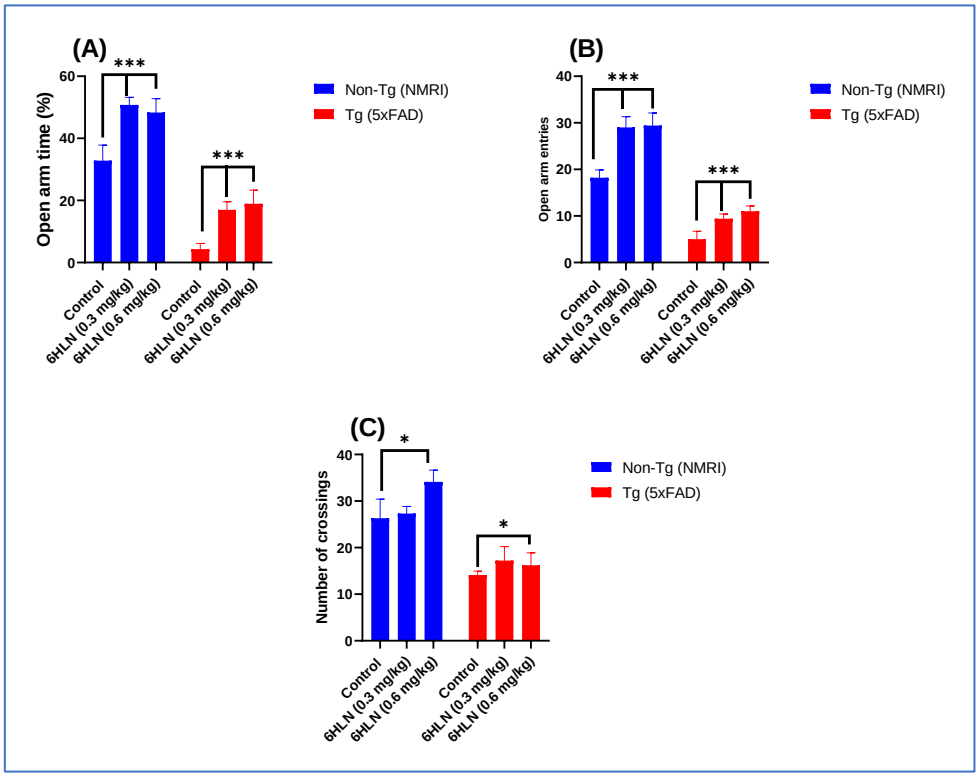

Fig. 5. Effects of 6-hydroxy-L-nicotine administration (6HLN, 0.3 and 0.6 mg/kg, g.c., i.p.) on the anxious response in the elevated plus-maze test. (A) Time spent in open arms; (B) Number of entries into open arms; (C) Number of open arm/closed arm transitions. NMRI mice and transgenic Tg (5xFAD) mice were used. Results are expressed as means ± E.S.M. (n=5), using two-way ANOVA followed by Tukey's post hoc test (*p<0.01 and ***p<0.0001).

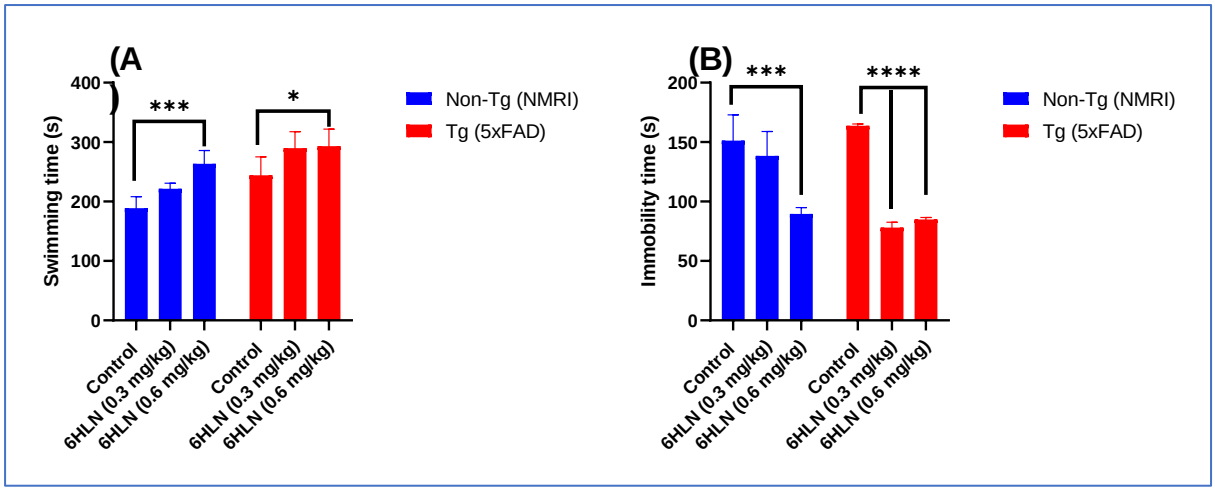

Fig. 6. Effects of 6-hydroxy-L-nicotine administration (6HLN, 0.3 and 0.6 mg/kg, g.c., i.p.) on the depressive response in the forced swimming test. (A) Swimming time (s); (B) Immobility time. NMRI mice and transgenic Tg (5xFAD) mice were used. Results are expressed as means ± E.S.M. (n=5), using two-way ANOVA followed by Tukey's post hoc test (*p<0.01, ***p<0.0001 and ****p<0.00001).

These results demonstrated that 6HLN exhibits significant effects on the level of Aβ1-42 aggregation, Aβ1-42-induced cytotoxicity (apoptosis), and Aβ1-42-induced memory deficits in the brains of 5xFAD transgenic animals.

Disseminating the results

In stage 2 of the project, the scientific results generated were published in a scientific article in the journal *Phytotherapy Research* (Q1, IF 7.2, AIS 1.25). Also, the scientific results were presented in 7 conferences (5 international and 2 national) in the form of 9 oral presentations and 2 posters. Participation in 2 workshops was also achieved.

The published article, participation in conferences and workshops were disseminated on the project web page:

http://cercetare.bio.uaic.ro/grupuri/bioactive/content/grants/PCE2022_hl.html.