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Research Article

Investigation of the pharmacological activity of the tetrapeptide HAEE, zinc, and human serum albumin in a transgenic mouse model with tau protein overexpression (P301S)

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Abstract

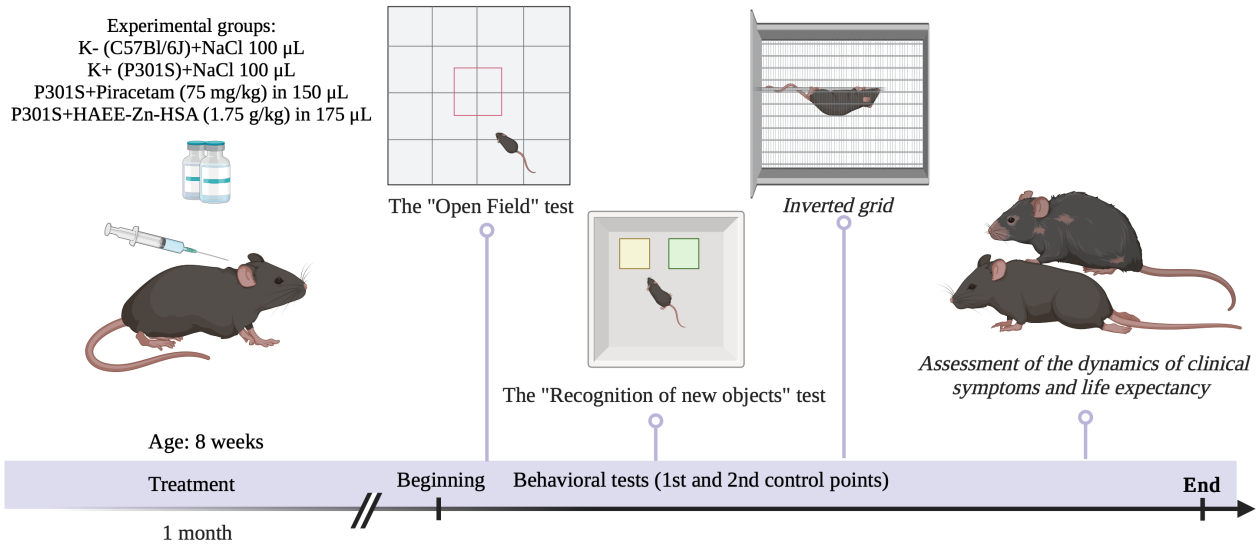
Introduction: Neurodegenerative diseases affecting neurons represent a wide spectrum of pathological conditions. To slow the accumulation of pathological tau protein, therapeutic interventions such as a pharmaceutical composition consisting of the tetrapeptide HAEE, zinc, and albumin may be employed. This composition possesses properties that can potentially slow disease progression.

Materials and Methods: The experiment was performed on 60 homozygous mice of both sexes from a transgenic line with the overexpression of human mutant tau protein (P301S) and on 20 mice from a wild-type C57Bl/6J background line. Control groups (C57Bl/6J – K and P301S – K+) received NaCl injection water (100 µL subcutaneously, once every 2 days); the experimental group (P301S) received HAEE-Zn-HSA (75 mg/kg, 150 µL subcutaneously, once every 2 days); and the comparison group (P301S) received piracetam (1.75 g/kg, 175 µL subcutaneously, once every 2 days). Behavioral activity was analyzed using tests at two time points, and the phenotypic presentation and lifespan were evaluated.

Results and Discussion: The P301S group of mice treated with HAEE-Zn-HSA at a dosage of 75 mg/kg showed positive behavioral activity in the following tests: Open Field, Novel Object Recognition, and Inverted grid test, indicating increased exploratory activity, and improved motor function and coordination in the mice of this group. Pharmacological intervention with HAEE-Zn-HSA in the experimental group of transgenic mice demonstrated positive results, as a statistically significant effect on lifespan and delayed onset of the phenotypic presentation of the disease was shown (24±2.14 weeks, $p \leq 0.05$).

Conclusion: The pharmaceutical composition HAEE-Zn-HSA at a dosage of 75 mg/kg, using a model of homozygous mice from a transgenic line with overexpression of human mutant tau protein P301S, demonstrated high levels of adaptive and exploratory activity and improved motor function.

Graphical abstract



Keywords

tauopathy, tau protein, behavioral activity, transgenic mice, HAEE-Zn-HSA complex pharmaceutical composition

Introduction

Alzheimer's disease (AD) is a common tauopathy characterized by memory loss and cognitive impairment. AD is an age-related neurodegenerative disease with two key hallmarks: extracellular amyloid plaques composed of amyloid-β (Aβ), and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated tau (Muralidar et al. 2020; Park et al. 2024).

The most prevalent post-translational modification of tau is phosphorylation, which affects its normal function, leading to microtubule destabilization. Furthermore, neurofibrillary tangles (NFTs) are a defining feature of Alzheimer's disease and other tauopathies. NFTs consist of hyperphosphorylated aggregates of tau protein (Sindi et al. 2024).

Autosomal dominant mutations in the *MAPT* gene, encoding tau, cause some forms of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), now termed FTDP-17t, demonstrating that tau dysfunction is sufficient for widespread neurodegeneration in the central nervous system. The pathology in humans with FTDP-17t is characterized by the presence of filamentous tau inclusions throughout the frontal and temporal lobes in neurons, and occasionally in glia, accompanied by atrophy in these regions, as well as ventricular enlargement. These *MAPT* mutations can cause various cognitive, behavioral, and motor deficits (Alquezar et al. 2021; Hurtle et al. 2024).

Homozygous transgenic mice with overexpression of human mutant tau protein (P301S) are often used as preclinical models for neurodegenerative diseases such as tauopathies (Deykin et al. 2021; Samudra et al. 2024; Stepenko et al. 2024). For treating tauopathies, interventions that slow the progressive accumulation of pathological tau protein, thereby mitigating disease progression, may be selected. A pharmaceutical composition based on the tetrapeptide HAEE (histidine, alanine, and 2 glutamic acids), zinc, and human serum albumin possesses such effects. Literature analysis has revealed that the synthetic peptide HAEE successfully binds to the metal-binding domain of amyloid β at the 11-14 region, thereby blocking zinc-induced dimerization of the domain and slowing the aggregation of full-length amyloid β in *in vitro* experiments (Kozin et al.

2019; Barykin et al. 2020). Importantly, the use of the HAEE-Zn-HSA complex significantly increases the bioavailability of the substance in the central nervous system, prolonging the half-life, which ultimately positively affects cognitive function in experimental animals. Under physiological conditions, the unique composition of ligands at the binding site is adapted and specific for zinc, allowing albumin to safely bind and deliver zinc with the necessary affinity (Szabo et al. 2023).

The aim of the study was to investigate the behavioral activity of homozygous mice from a transgenic line with overexpression of human mutant tau protein (P301S) following treatment with the HAEE-Zn-HSA complex pharmaceutical composition.

Materials and Methods

Animals

The study was conducted on 60 homozygous individuals of both sexes from a transgenic mouse line with overexpression of the human mutant Tau gene (P301S) and on 20 of a wild-type C57Bl/6J background line, also of both sexes, eight weeks of age. The animals were housed in the experimental biological clinic (SPF vivarium) of Belgorod State National Research University (BelSU). All experimental studies and manipulations on animals were performed in accordance with the ethically acceptable standards and approved by the Bioethics Committee of BelSU (minutes №. 15/10 dated October 29, 2021).

Experimental design

The study included four groups of animals, with 20 mice of both sexes in each group:

Control groups C- C57Bl/6J and C+ P301S 20: animals were injected with NaCl (100 µL subcutaneously, once every 2 days);

Experimental group P301S: animals were injected with HAEE-Zn-HSA (75 mg/kg, in a volume of 150 µL subcutaneously, once every 2 days);

Comparison group P301S: animals were injected with piracetam (1.75 g/kg, in a volume of 175 µL subcutaneously, once every 2 days).

Behavioral activity assessment

The study of behavioral activity was carried out at two control time points, the first at 3 months of age and the second at 5 months of age.

Open field test

This test assesses the behavioral activity of animals, such as exploratory, defensive, and motor activity. All these data are investigated by measuring the speed of movement of the mouse, the distance traveled, and the time spent in the center and periphery of the field. Mice were placed in the center of the arena (OpenScience, Russia) measuring 50x50 cm, with a field height of 40 cm and uniform field illumination of 40 lux. A video camera was located above the arena under the ceiling, recording the animal's movements in the arena. The animal remained in the arena for 5 minutes.

Novel object recognition test

This test is used to analyze the spontaneous tendency of mice to exhibit a greater number of interactions with a novel object than with a familiar object. During the habituation phase, each mouse was allowed to freely explore the open field arena (a white box 40 cm wide, 40 cm deep, and 40 cm high) without any objects present. The mouse was then removed from the box and placed in its cage. During the familiarization period, each mouse was placed in the white box with two identical objects for 5 minutes and quickly returned to the cage. Recognition memory was tested 24 hours later, when the mouse was exposed to one familiar object and one novel object.

Inverted grid test

This test allows for the assessment of neuromuscular strength and coordination in mice over a period of 1 minute. A mouse was placed in the center of a 20cm² wire mesh with a barrier along the edge, and then the mesh was inverted. The time spent in contact with the mesh was recorded. Each animal was tested in three separate trials with a 10-minute interval between each trial. The average time spent in contact with the mesh is reported.

Evaluation of clinical symptom progression and lifespan

To investigate the effect of HAEE-Zn-HSA on the lifespan of mice with tau proteinopathy from the P301S transgenic line, 60 males and females were selected. Animals were randomized into two groups: the first group of animals received subcutaneous injections of HAEE-Zn-HSA at a dose of 75 mg/kg every other day, starting from day 60 of life and continuing for 27 days. The second group of animals received a control solution consisting of 150 µL of water for injection, subcutaneously every other day for 27 days. Neurological monitoring was performed to detect the first symptoms of hind limb paresis. The nature of data distribution was analyzed using the Shapiro-Wilk method. The significance of differences between the groups was assessed using the Student's t-test for the age of the animals at the appearance of the first phenotypic signs of the disease and the time for the development of a complete neurological deficit.

Statistical analysis

All the obtained results of behavioral activity were subjected to statistical processing using GraphPad Prism 8.0 software (California, USA); a two-way ANOVA followed by Tukey's *post hoc* test was used. The normality of the distribution was checked using the Kolmogorov-Smirnov test (n = 10 mice) $p<0.01$; $p<0.001$; $p<0.0001$; ns.

Results and Discussion

Results of behavioral activity in the Open Field Test

At the first control time point, the third group of P301S mice with piracetam pharmacotherapy showed moderate speed and distance traveled in the apparatus, but exhibited a slight increase in the number of entries into the center and the time spent in this zone, thereby increasing the indicators of being in the periphery and the time that mice spent in this zone. It means that mice in this group had moderate adaptive activity of 63.78 ± 8.65%; these indicators are higher than those of the P301S group without treatment ($p < 0.0001$), but lower than those of the control group C57Bl/6J ($p < 0.0001$). Meanwhile, the fourth group of P301S mice, with HAEE-Zn-HSA pharmacotherapy, demonstrated reduced anxiety levels and also showed rapid movement and distance traveled in the studied zones, an increase in the number of entries into the center and the time spent in this zone, and, conversely, a decrease in being in the periphery and the time spent in this zone, which indicates high adaptive activity of 72.93 ± 3.35% ($p < 0.0001$) compared to the those in the positive control group P301S without treatment (Fig. 1).

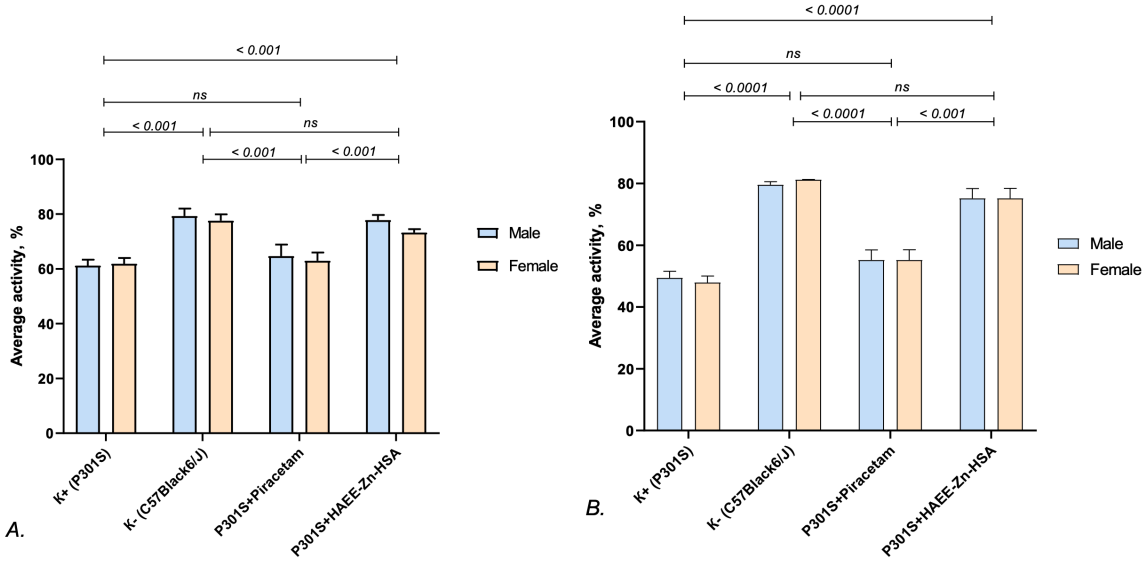


Figure 1. Results of the Open Field Test following treatment with HAEE-Zn-HSA (75 mg/kg) and piracetam (1.75 g/kg). ***Note*: A** – average activity levels at the first time point (mice aged 3 months); **B** – average activity levels at the second time point (mice aged 5 months).

At the second control time point, the positive control group also exhibited low speed and distance traveled, as well as indicators of being in the central zone and the time spent in the center, but there was an increase in being in the periphery of the open field and the time spent in this zone increased. The absence of improvement in adaptive activity, 48.66 ± 5.07% ($p < 0.0001$) compared with the negative control, indicates the chronic nature of pathological changes in the brains of mice with tauopathy. It is worth noting that the group of P301S mice with HAEE-Zn-HSA pharmacotherapy showed reduced indicators of anxiety levels and also rapid movement and distance traveled in the studied zones. Adaptive activity indicators significantly increased in this group, and amounted to 36%, $p < 0.001$ compared to the group receiving piracetam.

Results of behavioral activity in the Novel Object Recognition (NOR) Test

At the first control time point, the results of the NOR test for the negative control group of C57Bl/6J mice showed high values for distance, speed, and indicators of interest in the novel object, which subsequently reflected in increased preference and discrimination indices (0.49 ± 0.16 units), and discrimination (0.59 ± 0.1 units), $p < 0.0001$ compared to the positive control. Meanwhile, the group of P301S mice with piracetam pharmacotherapy showed a moderate increase in the parameters of distance, speed, whereas indicators of interest in the novel object (41 ± 2.83 units) and the values of the preference indices (0.4 ± 0.2 units) and discrimination (0.41 ± 0.16 units) were low, which may indicate a reduced recognition of the novel object. The group of P301S mice with HAEE-Zn-HSA pharmacotherapy showed that the values of the preference indices (0.45±0.18 units) and discrimination (0.56±0.15 units) compared to the positive control $p<0.0001$ and the group receiving piracetam $p < 0.01$ (Fig. 2).

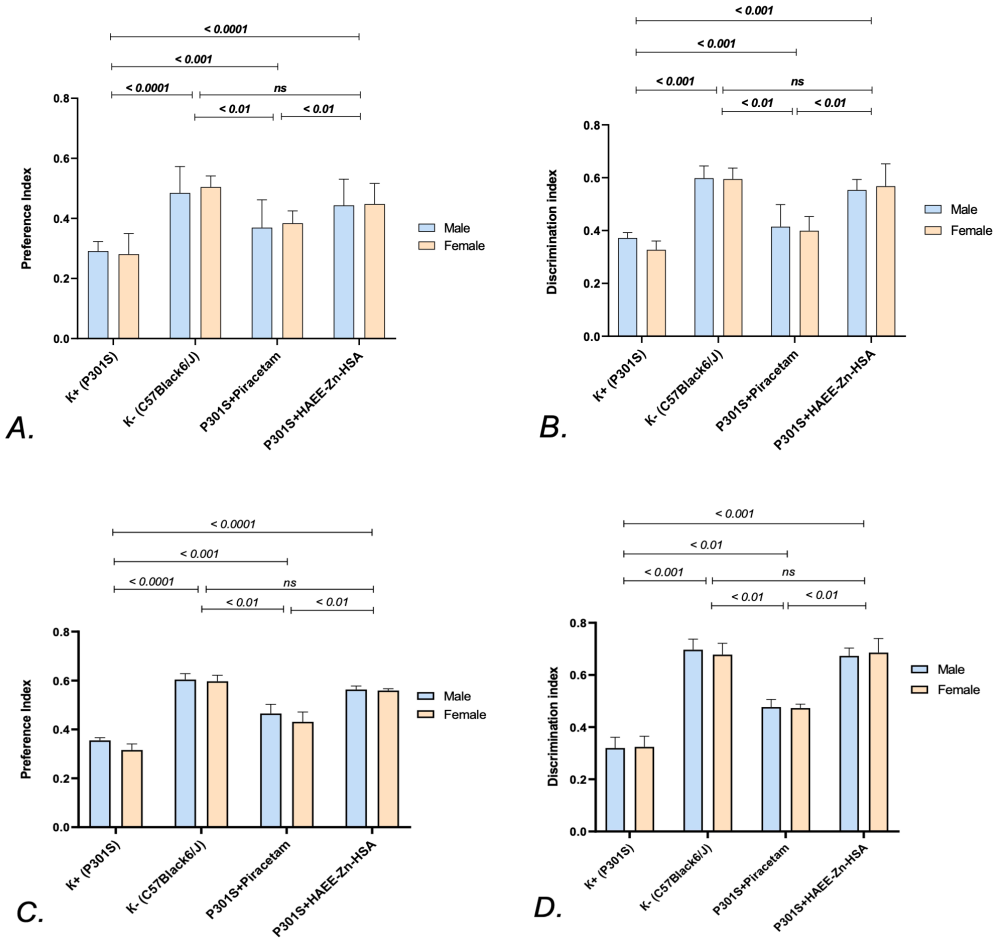


Figure 2. Novel Object Recognition Test with HAEE-Zn-HSA (75 mg/kg), piracetam (1.75 g/kg). ***Note:*** **A** – results at Timepoint 1, Preference Index (mice aged 3 months), **B** – results at Timepoint 1, Discrimination Index (mice aged 3 months), **C** – results at Timepoint 2, Preference Index (mice aged 5 months), **D** – results at Timepoint 2, Discrimination Index (mice aged 5 months).

At the second control time point, the positive control group showed a decrease in distance, speed and indicators of interest in the novel object, as well as values of the preference indices (0.34 ± 0.05 units) and discrimination (0.32 ± 0.09 units), $p < 0.0001$ were low compared to the negative control group and the treatment groups, which indicates the presence of a cognitive deficit in these mice. Meanwhile, the negative control showed high values for the parameters of distance, speed, indicators of interest in the novel object, and also high values of the preference indices (0.600 ± 0.057 units) and discrimination (0.68 ± 0.1 units), indicating the absence of cognitive deficits in the control group mice. The group of P301S mice with piracetam pharmacotherapy demonstrated low values of preference indices (0.448 ± 0.092 units) and discrimination (0.48 ± 0.05 units), $p < 0.001$ compared to the positive control group, and the group of P301S mice with HAEE-Zn-HSA pharmacotherapy also continued to show high parameter values of preference indices (0.561 ± 0.027 units) and discrimination (0.68 ± 0.1 units), which indicates a possible neuroprotective effect of the pharmaceutical composition.

Results of behavioral activity in the Inverted Grid Test at the First and Second Control Points

In the positive control group, P301S mice without treatment showed severe impairments in motor coordination and motor function. This was confirmed by low values indicating the time of maintaining balance on the inverted grid, where the average values for the first control point and second control time points were 38.91 ± 9.49 s and 7.94 ± 4.58 s, respectively, compared to the negative control group (Fig. 3).

In the negative control group C57Bl/6, mice in this group coped with the inverted grid retention test without any problems throughout the testing period, where the average values for the first and second control time points were 51.86±14.15 s and 54.12±12.25 s, respectively, which confirms the absence of motor function and coordination impairments compared to the positive control ($p < 0.0001$).

In the comparison group of P301S mice receiving piracetam, a two-fold decrease in retention indicators was shown at the second control time point (41.07 ± 14.14 s; 21.46 ± 6 s) compared to the negative control group ($p < 0.0001$) and the group receiving HAEE-Zn-HSA ($p < 0.0001$).

In the experimental group of P301S mice receiving HAEE-Zn-HSA, an improvement in balance retention indicators on the inverted grid was shown at both control time points: 51.29 ± 8.43 s and 38.04 ± 12.1 s, compared to the positive control group ($p < 0.001$) and the group receiving piracetam ($p < 0.0001$). This is confirmed by improved motor functions and coordination abilities.

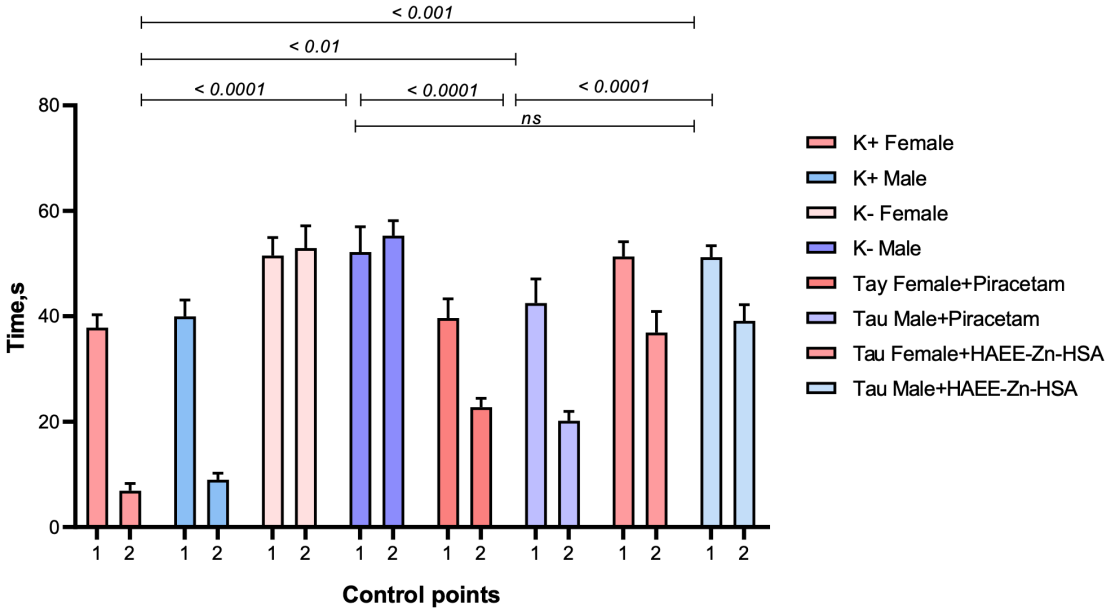


Figure 3. Inverted Grid Test: effect of HAEE-Zn-HSA (75 mg/kg) and piracetam (1.75 g/kg) at the first (mice aged 3 months) and second (mice aged 5 months) control time points.

Assessment of clinical symptom progression and lifespan

In transgenic mice of the P301S line, expressing an aberrant tau protein gene with a homozygous genotype, the phenotypic picture begins to manifest at 16 weeks of age, and at 22 weeks the pronounced symptoms reach their peak (weight loss, hunched posture, bilateral paralysis of the hind limbs, decreased corneal reflex and pinna reflex). In the positive control group (P301S) receiving sodium chloride solution in an equivalent amount, the lifespan was 20.7 ± 1.9 weeks, where $p \leq 0.05$ according to Student's t-test. Pharmacological correction in the experimental group of transgenic mice using HAEE-Zn-HSA showed positive results, as the effect on lifespan and delay in the onset of the phenotypic picture of the disease was statistically proven to be 24±2.14 weeks, where $p \leq 0.05$ according to Student's t-test (Fig. 4).

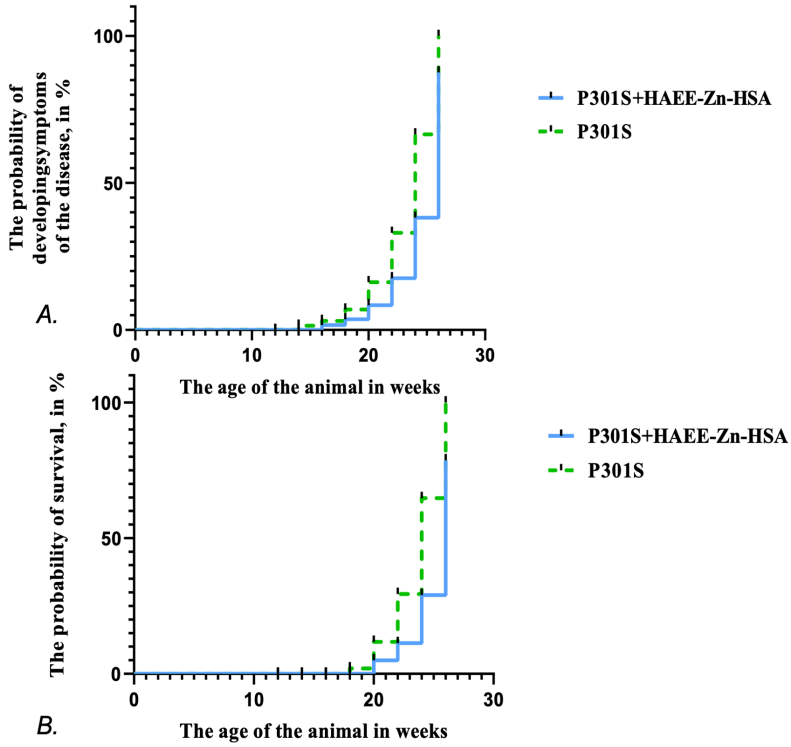


Figure 4. Evaluation of HAEE-Zn-HSA (75 mg/kg) Pharmacocorrection on lifespan and dynamics of clinical symptom onset in tau proteinopathy in transgenic P301S mice. ***Note:*** **A** – Kaplan-Meier Curve showing the probability of developing disease symptoms in the transgenic P301S mouse line; **B** – Kaplan-Meier Curve showing the probability of lifespan in the transgenic P301S mouse line. Statistical analysis was performed using the Kaplan-Meier method with the Mantel-Cox log-rank test.

Conclusion

The results obtained in this study showed that HAEE-Zn-HSA at a dosage of 75 mg/kg demonstrated a more pronounced neuroprotective effect compared to piracetam. The results of the behavioral tests (Open Field, Novel Object Recognition) showed improved adaptive activity and cognitive function in mice treated with HAEE-Zn-HSA (75 mg/kg) compared to the control group and the group treated with piracetam. Mice treated with HAEE-Zn-HSA (75 mg/kg) exhibited less anxiety and adapted more quickly to a new environment. In the Inverted Grid test, mice treated with HAEE-Zn-HSA (75 mg/kg) demonstrated improved motor coordination and motor function.

Representative indicators from the assessment of clinical symptom progression and lifespan indicate a statistically significant increase in lifespan and a delay in the onset of disease symptoms in mice treated with HAEE-Zn-HSA (75 mg/kg) compared to the positive control group.

Additional information

Conflict of interest

The authors declare no conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

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