

Possible Ameliorative Potentials of Pentoxifylline Against Alzheimer's Disease Induced in a Rat Model

Posibles Efectos Beneficiosos de la Pentoxifilina Contra la Enfermedad de Alzheimer Inducida en un Modelo de Rata

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SUMMARY: Alzheimer's disease is the most common cause of dementia, and no current therapy reverses the underlying neurodegeneration. In rats, chronic aluminum chloride (AlCl₃) reproduces the core lesions of the disease: amyloid- β deposition, tau hyperphosphorylation, reactive astrogliosis, and hippocampal neuronal loss. Pentoxifylline (PTX) is an oral phosphodiesterase inhibitor with anti-inflammatory, antioxidant, and hemorheological actions, making it a plausible candidate for repurposing. Forty male Wistar rats were divided into four equal groups (n = 10): vehicle, PTX, AlCl₃, and AlCl₃ + PTX. AlCl₃ (100 mg/kg/day, i.p.) and PTX (10 mg/kg/day, i.p.) were given for 60 days. We assessed histology and immunohistochemistry (A β 42, AT8, GFAP), hippocampal ELISA panels, and qRT-PCR for BAX and Bcl-2, and compared groups by one-way ANOVA with Tukey's HSD. AlCl₃ produced severe hippocampal pathology. Neuronal density fell by 64 %, the pyknotic index reached 72 %, Congo red revealed about 11 birefringent amyloid plaques per field, and the GFAP area fraction rose almost fourfold; TNF- α , IL-6, and ICAM-1 rose 1.9- to 4.1-fold. Tissue A β 42 climbed 4.6-fold, acetylcholine fell to 23 % of control, and the BAX/Bcl-2 ratio more than doubled. PTX shifted every parameter back toward normal (p < 0.001): amyloid burden dropped 58–68 %, phospho-tau 56–59 %, astrogliosis 40–43 %, and the cognitive deficits 46–48 %. TNF- α and MDA returned to control values. PTX produced substantial, though partial, neuroprotection. The pattern fits the drug's known pharmacology, but the study is hypothesis-generating rather than confirmatory: it used male rats only, a concurrent rather than delayed treatment schedule, and a single dose. Delayed-treatment, both-sex, and dose–response studies are needed before any clinical step.

KEY WORDS: Alzheimer disease; Amyloid beta-peptides; Tau proteins; Gliosis; Oxidative stress; Pentoxifylline.

INTRODUCTION

Alzheimer's disease affects more than 55 million people worldwide, a figure projected to nearly triple by 2050 (WHO, 2021). Amyloid- β plaques, hyperphosphorylated tau tangles, neuronal loss, synaptic degeneration, and chronic neuroinflammation together dismantle the hippocampal and cortical circuits that support memory and executive function (Heneka *et al.*, 2010; Exley, 2017). No approved therapy halts or reverses that process. The anti-amyloid antibodies lecanemab and donanemab help modestly in early disease, but they carry cerebrovascular risks, cost a great deal, and remain out of reach for most patients (van Dyck *et al.*, 2023). That gap is what keeps the search for repurposable, already-safe drugs alive.

Chronic AlCl₃ dosing in rats reproduces much of the histopathology of human Alzheimer's disease - amyloid deposits, neurofibrillary tangle-like changes, neuronal loss, reactive astrogliosis, and oxidative stress - through mechanisms that partly overlap with the human disease (Liaquat *et al.*, 2019; Islam *et al.*, 2022; Mustafa, 2020). Pentoxifylline has been in clinical use for four decades, and its pharmacology maps onto several of these processes: it inhibits PDE4 and PDE5, raises intracellular cAMP, dampens NF- κ B-driven inflammation, lowers TNF- α and IL-1 β , blunts oxidative stress, improves cerebral microvascular flow, and supports CREB-dependent neurotrophic signaling (Neves *et al.*, 2015; Wang *et al.*, 2020; Khalifa *et al.*, 2022).

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Here we test PTX in the AlCl_3 model using histology, immunohistochemistry ($\text{A}\beta 42$, AT8), hippocampal ELISAs, and qRT-PCR for BAX and Bcl-2. Because PTX was given alongside AlCl_3 rather than after the pathology was established, what the study measures is neuroprotection rather than reversal.

MATERIAL AND METHOD

Ethical Approval

This study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, King Abdulaziz University (Reference No. 213-19) and by the Institutional Animal Care and Use Committee (IACUC No. IACUC-2023/BM-041). Procedures followed the NIH Guide for the Care and Use of Laboratory Animals (National Research Council, 2011); reporting follows the ARRIVE 2.0 guidelines (Percie du Sert *et al.*, 2020).

Animals

Forty male Wistar albino rats (7 weeks old, 200–250 g) were obtained from a specific-pathogen-free facility. Only males were used to avoid hormonal variability that can confound oxidative-stress and amyloid-related outcomes (Tozlu *et al.*, 2022); future work should include females. Animals were group-housed (five per cage) at $22 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ humidity under a 12-h light/dark cycle, with pellet diet and filtered water *ad libitum*, and acclimatized for one week before any intervention.

Chemicals

Aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$; Cat. No. 8010810500; Merck Millipore/Sigma; purity $\geq 99\%$) and pentoxifylline (Sigma-Aldrich; purity $\geq 98\%$) were used. PTX was dissolved in sterile saline at 200 mM and stored at -4°C for no longer than four days. Solutions were prepared fresh and dosed by morning body weight.

Experimental Design

Animals were randomly allocated by computer-generated block randomization into four groups of ten. Allocation, histological scoring, and behavioral scoring were each done by separate observers blinded to group. A second observer re-scored 20 % of fields blind to both group and primary score; intraclass correlation coefficients exceeded 0.85 across all parameters.

Group 1 - Control: sterile saline (i.p.) once daily for 60 days at a volume matched to the AlCl_3 and PTX groups.

Group 2 - PTX Only: PTX 10 mg/kg/day, i.p., for 60 days (Neves *et al.*, 2015).

Group 3 - AD Model: AlCl_3 100 mg/kg/day, i.p., for 60 days, following reported neurotoxic dosing (Mustafa, 2020). This dose is widely used to model aluminum neurotoxicity in rats and is far above human exposure levels.

Group 4 - AD + PTX: AlCl_3 (100 mg/kg/day) plus PTX (10 mg/kg/day), both i.p., over the same 60-day period.

Body weight was recorded at baseline and on days 15, 30, 45, and 60, with general behavior noted at each weighing. All 40 animals completed the experiment.

Behavioral Assessment

Testing was carried out in the final week of treatment (days 56–60) during the light phase (09:00–13:00) at $22 \pm 2^\circ\text{C}$ and 40–60 lux. Animals were habituated to the testing room for 30 min before each session, and apparatus surfaces were cleaned with 70 % ethanol between animals.

Morris Water Maze

A circular tank (150 cm diameter, 60 cm high) was filled with opaque water ($25 \pm 1^\circ\text{C}$, white tempera) to a depth of 30 cm, with a 12-cm escape platform submerged 1.5 cm below the surface in the center of a fixed target quadrant (Morris, 1984). Each animal completed four acquisition trials per day for five days (90 s maximum, 20-min inter-trial interval). Escape latency and path length were captured with EthoVision XT 15.0 (Noldus). On the probe day the platform was removed and animals explored freely for 60 s; target-quadrant dwell time and platform-location crossings were recorded.

Novel Object Recognition

The arena was a white PVC open-field box ($60 \times 60 \times 40$ cm). After 10-min habituation, animals had a 10-min acquisition session with two identical objects, followed 24 h later by a 10-min test with one familiar object replaced by a novel object (Ennaceur & Delacour, 1988). Exploration was defined as directed sniffing or touching within 2 cm. Discrimination index ($\text{DI} = [\text{novel} - \text{familiar}] / [\text{novel} + \text{familiar}]$) and recognition index ($\text{RI} = \text{novel} / [\text{novel} + \text{familiar}]$) were calculated.

Passive Avoidance

The apparatus had two equal compartments (20×20

× 20 cm), one lighted and one dark, separated by a guillotine door (8 × 8 cm). The dark side had a stainless-steel grid floor wired to an isolated stimulator. During acquisition, animals received an intermittent foot shock (50 Hz, 1.5 mA, 5 s) on entering the dark compartment, and training ended when the animal stayed in the lighted compartment for 120 consecutive seconds. Retention was tested 24 h later: step-through latency and time spent in the dark compartment were recorded, with a 300-s ceiling for non-crossers (Ambrogio Lorenzini *et al.*, 1996).

Tissue Collection

After the behavioral battery, animals were deeply anaesthetized with chloral hydrate (400 mg/kg, i.p.). For histology, animals were transcardially perfused with ice-cold PBS (0.1 M, pH 7.4) followed by 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). For biochemistry and gene expression, brains were rapidly removed, hippocampi dissected on ice in artificial CSF, snap-frozen in liquid nitrogen, and stored at -80 °C. Postfixed brains (24–72 h at 4 °C) were dissected stereotactically, paraffin-embedded after graded ethanol dehydration and xylene clearing (Leica TP1020), and cut at 5 µm on a Leica RM2235 microtome. Parallel sections at matched levels were reserved for each staining protocol so all stains could be compared within the same animal.

Histological and Immunohistochemical Staining

Haematoxylin and Eosin

Deparaffinized sections were rehydrated, stained with Harris haematoxylin (8 min), differentiated in 1 % acid alcohol (30 s), blued in tap water (5 min), counterstained with 1 % aqueous eosin-phloxine (3 min), dehydrated, cleared, and mounted with DPX. Slides were reviewed by two board-certified histologists blinded to group.

Modified Bielschowsky Silver Impregnation

Deparaffinized sections were incubated in 20 % silver nitrate at 37 °C for 30 min in the dark, then in ammoniacal silver, washed in distilled water, and developed in a formaldehyde-based reducing solution under microscopic monitoring until brown-black impregnation was optimal. Sections were toned in 0.2 % gold chloride, fixed in 5 % sodium thiosulfate, dehydrated, cleared, and mounted with DPX. All steps within a batch were run together to eliminate inter-run variability.

Congo Red

Hydrated sections were stained in freshly prepared alkaline Congo red (1 % NaCl, pH 10.2, with 0.5 % Congo

red; Sigma-Aldrich) for 20 min at room temperature, briefly differentiated in alkaline alcohol (1 % NaOH in 80 % ethanol, 10–20 s), counterstained with Mayer's haematoxylin (2 min), and mounted with DPX. Slides were examined under both bright-field and cross-polarized light. Apple-green birefringence was required for definitive amyloid classification (Sunde & Blake, 1998; Howie & Brewer, 2009). Known amyloid-positive and amyloid-negative tissue served as controls in every run.

GFAP Immunohistochemistry

Heat-induced antigen retrieval used sodium citrate buffer (10 mM, pH 6.0) at 95 °C for 20 min. Endogenous peroxidase was quenched with 3 % H₂O₂ in methanol (15 min) and non-specific binding blocked with 5 % normal serum (30 min). Sections were incubated overnight at 4 °C with polyclonal rabbit anti-GFAP (1:400; Abcam), then with biotinylated secondary antibody and streptavidin-HRP. DAB was used for visualization, with Mayer's haematoxylin counterstain. Negative controls (non-immune serum) were processed in every run.

Amyloid-β42 Immunohistochemistry

After heat-induced retrieval in sodium citrate buffer (10 mM, pH 6.0) at 95 °C for 20 min, sections were incubated overnight at 4 °C with rabbit monoclonal anti-Aβ42 (clone mOC64; 1:200; Abcam, ab201060), then with biotinylated goat anti-rabbit secondary antibody and streptavidin-HRP (VECTASTAIN Elite ABC HRP Kit, Vector Laboratories). DAB visualization and Mayer's haematoxylin counterstain were used. Negative controls (non-immune rabbit IgG) and positive controls (known human Alzheimer's disease cortex) were processed alongside experimental sections.

Phospho-tau (AT8) Immunohistochemistry

AT8 recognizes tau phosphorylated at Ser202/Thr205, the earliest detectable hyperphosphorylation event in human Alzheimer's disease neurofibrillary tangle formation (Braak *et al.*, 2006). After retrieval in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 9.0) at 95 °C for 20 min, sections were incubated overnight at 4 °C with mouse monoclonal anti-phospho-tau AT8 (1:100; Thermo Fisher MN1020), then with anti-mouse biotinylated secondary antibody and streptavidin-HRP. DAB visualization and Mayer's haematoxylin counterstain were used. Positive controls used known tauopathy-positive rat hippocampus from the same run.

Biochemical Analyses

Hippocampi were homogenized in 1.15 % KCl at

1:10 (w/v) and centrifuged at 1,000 rpm for 1 min at 4 °C; supernatants were used. For TNF- α , tissue was homogenized in ice-cold RIPA lysis buffer with protease and phosphatase inhibitors (Sigma-Aldrich) at 1:10 (w/v) and centrifuged at 12,000 $\times$ g for 15 min at 4 °C. Total protein was quantified by BCA assay (Pierce, Thermo Fisher) against a BSA standard curve.

A β 42, acetylcholine, and acetylcholinesterase were quantified by ELISA (Rat Amyloid beta 42, MBS457329; Rat Acetylcholine, MBS728879; Rat Acetylcholinesterase, MBS2508152; all MyBioSource). TNF- α (RayBio RAB0479), IL-6 (RayBio ELR-IL6-001), and ICAM-1 (RayBio ELR-ICAM1) were quantified by ELISA, with intra- and inter-assay CVs below 8 % and 12 %. Reduced glutathione (MBS046356), malondialdehyde (MBS738685), and superoxide dismutase 3 (MBS4502589) were also measured by ELISA (My BioSource). All assays followed manufacturer instructions; results are reported per gram of brain tissue.

BAX and Bcl-2 qRT-PCR

Total RNA was extracted with the RNeasy Mini Kit (Cat. No. 61074, QIAGEN), eluted in 40 μ L nuclease-free water, and stored at -20 °C. RNA purity (A260/A280) was checked spectrophotometrically. Equal amounts of RNA were reverse-transcribed with a commercial kit, and qPCR used a SYBR Green assay on a Rotor-Gene Q 5-Plex Real-Time Rotary Analyzer (Corbett Life Science). Each 25- μ L reaction contained 2 μ L cDNA, 12.5 μ L SYBR Green Master Mix, 0.63 μ L Hot Start Taq polymerase, 1 μ L internal control, 0.1 μ L uracil-DNA glycosylase, and nuclease-free water to volume. Every run included no-template controls and reference standards.

Cycling: 50 °C for 2 min; 95 °C for 10 min; 40 cycles of 95 °C/15 s, 57 °C/45 s, 72 °C/45 s; then 60 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min. Fluorescence was acquired at 60 °C, with FAM (F1) for BAX/Bcl-2 and HEX (F2) for the internal control. For Bcl-2, four positive reference standards (1×10^7 , 1×10^6 , 1×10^5 , and 1×10^4 IU/mL) were included. Relative expression was calculated by the comparative Ct ($2^{-\Delta\Delta C_t}$) method against an internal reference gene. Samples were run in triplicate.

Morphometric Analysis

Stained sections were examined on an Olympus BX53 microscope with a calibrated Olympus DP74 camera. Images were captured at $\times 200$ (H&E, silver, Congo red) and $\times 400$ (GFAP). Five randomly chosen non-

overlapping fields per section from three non-consecutive sections per animal were analyzed, following systematic uniform random sampling (Mustafa, 2021). Measurements used calibrated ImageJ v.1.54 (NIH) by an observer blinded to group; a second observer re-analyzed 20 % of fields, with intraclass correlation coefficients of 0.85–0.96. The biological replicate was the individual animal; the 15 fields per animal were averaged before any between-group comparison.

Statistical Analysis

Sample size was determined a priori using G*Power v.3.1 with Cohen's $d = 1.2$, giving $n \geq 6$ per group for histology and $n = 10$ for biochemistry and gene expression at ≥ 80 % power and $\alpha = 0.05$. Data are mean $\pm$ SD (or SEM where noted). Normality (Shapiro–Wilk) and variance homogeneity (Levene) were checked before parametric testing. Groups were compared by one-way ANOVA followed by Tukey's HSD, with family-wise error rate controlled at $\alpha = 0.05$; groups sharing a superscript letter were not significantly different. MWM acquisition data were analyzed with a linear mixed-effects model (fixed: Group, Day, Group $\times$ Day; random: animal) and Bonferroni-corrected post-hoc tests. Ordinal scores used Kruskal–Wallis with Dunn's correction. Effect sizes are partial η^2 . Analyses were run in SPSS v.29.0 and GraphPad Prism v.10.2; significance was set at $p < 0.05$, with values below detection limits reported as $p < 0.001$.

RESULTS

General Observations

All 40 animals survived the 60-day protocol. Body weight stayed stable in controls and PTX-only animals. From around day 14, AlCl₃-treated animals (Group 3) showed progressive behavioral decline: reduced locomotor activity, less grooming, sluggish responses, and lower body weight, consistent with aluminum-induced systemic neurotoxicity (Liaquat *et al.*, 2019). Group 4 animals were noticeably less affected, with several maintaining near-normal activity into the final week.

H&E Morphometric Analysis

Control and PTX-only sections had compact orderly laminar bands, open vesicular nuclei with prominent nucleoli, and pale cytoplasm. No vacuolation, amyloid deposits, or inflammatory change was visible, and PTX-only sections matched controls on every parameter ($p > 0.05$). AlCl₃-treated sections showed severe neurodegeneration: neuronal density fell 64 %, the pyknotic

index reached 72 %, plaque-like deposits averaged 8.6/field, and band integrity collapsed to 22 %. PTX co-treatment improved every H&E parameter ($p < 0.001$ vs AD): neuronal density nearly doubled, the pyknotic index

fell to 28 %, plaque-like deposits dropped about 73 %, and band integrity tripled. All values stayed below control, so the neuroprotection is partial, not complete (Table I, Fig. 1).

Table I. Morphometric parameters of hippocampal H&E sections (mean $\pm$ SD; $n = 10$ /group; one-way ANOVA + Tukey HSD). Different superscripts (a, b, c) indicate significant between-group differences ($p < 0.05$).

H&E Parameter	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
Neuronal density (cells/mm ²)	181.4 $\pm$ 11.8 ^a	176.2 $\pm$ 14.3 ^a	64.7 $\pm$ 17.5 ^c	127.9 $\pm$ 21.4 ^b	89.47	<0.001
Mean nuclear diameter (μ m)	8.52 $\pm$ 0.87 ^a	8.31 $\pm$ 0.94 ^a	5.08 $\pm$ 1.38 ^c	7.19 $\pm$ 1.12 ^b	54.31	<0.001
Pyknotic neuron index (%)	3.1 $\pm$ 1.0 ^a	4.0 $\pm$ 1.2 ^a	72.4 $\pm$ 8.9 ^c	27.8 $\pm$ 7.1 ^b	312.6	<0.001
Amyloid-like plaque count (/field)	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	8.6 $\pm$ 2.0 ^c	2.3 $\pm$ 1.1 ^b	187.4	<0.001
Neuropil vacuolation score (0–3)	0.2 $\pm$ 0.1 ^a	0.3 $\pm$ 0.2 ^a	2.9 $\pm$ 0.2 ^c	1.5 $\pm$ 0.4 ^b	421.8	<0.001
Perineuronal space width (μ m)	0.8 $\pm$ 0.2 ^a	0.9 $\pm$ 0.3 ^a	4.3 $\pm$ 0.9 ^c	2.2 $\pm$ 0.6 ^b	178.3	<0.001
Band organizational integrity (%)	96.4 $\pm$ 2.1 ^a	94.8 $\pm$ 3.2 ^a	22.3 $\pm$ 8.7 ^c	67.5 $\pm$ 12.4 ^b	245.7	<0.001

Neuropil vacuolation: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Bars: mean $\pm$ SD; $n = 10$ /group. Letters (a, b, c) mark groups that differ at $p < 0.05$ (Tukey HSD).

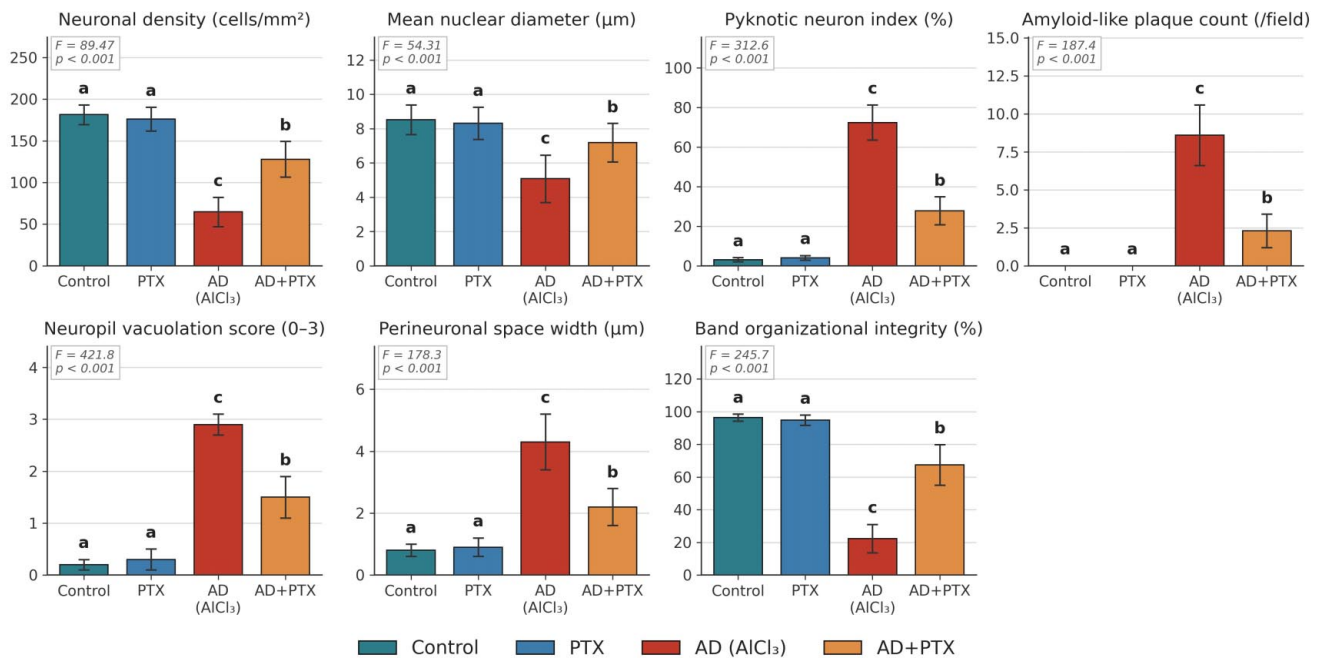


Fig. 1. Hippocampal H&E morphometry by group (Table I). Bars are mean $\pm$ SD ($n = 10$); letters (a, b, c) mark groups differing at $p < 0.05$ by one-way ANOVA with Tukey's HSD.

Silver Stain (Bielschowsky) Morphometric Analysis

Silver-stained control sections had a pale background with minimal impregnation, and PTX-only sections looked the same. AlCl₃-treated sections carried severe argentophilic pathology: optical density rose fivefold, silver-positive area expanded tenfold, NFT-like

structures reached around 19/field, and the neuronal loss index hit 47 %. PTX co-treatment produced an intermediate profile: optical density fell 47 %, plaque-like deposits 59 %, NFT-like structures 59 %, and the neuronal loss index halved to 23 % (Table II, Fig. 2).

Table II. Morphometric parameters of brain tissue sections with modified Bielschowsky silver impregnation (mean $\pm$ SEM; $n = 10$ /group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences ($p < 0.05$).

Silver Stain Parameter	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
Mean optical density of silver staining (AU)	28.4 $\pm$ 2.1 ^c	31.7 $\pm$ 2.6 ^c	143.2 $\pm$ 8.9 ^a	76.5 $\pm$ 5.3 ^b	—	<0.001
Silver-positive neurons (cells/mm ²)	44.8 $\pm$ 3.7 ^c	46.3 $\pm$ 4.0 ^c	157.6 $\pm$ 12.4 ^a	88.9 $\pm$ 7.1 ^b	—	<0.001
Area of silver-positive deposits (%)	4.1 $\pm$ 0.7 ^c	4.9 $\pm$ 0.8 ^c	39.2 $\pm$ 3.4 ^a	18.7 $\pm$ 2.2 ^b	—	<0.001
Amyloid plaque-like deposits (/field)	0.9 $\pm$ 0.3 ^c	1.2 $\pm$ 0.4 ^c	13.1 $\pm$ 1.9 ^a	5.4 $\pm$ 1.0 ^b	—	<0.001
Mean neuronal perikaryon diameter (μ m)	12.6 $\pm$ 0.9 ^a	12.3 $\pm$ 0.8 ^a	8.4 $\pm$ 0.7 ^b	10.8 $\pm$ 0.6 ^c	—	<0.001
NFT-like structures (/field)	1.1 $\pm$ 0.4 ^c	1.3 $\pm$ 0.5 ^c	19.4 $\pm$ 2.3 ^a	7.9 $\pm$ 1.3 ^b	—	<0.001
Neuronal loss index (%)	0 (ref) ^c	2.1 $\pm$ 0.6 ^c	47.3 $\pm$ 4.8 ^a	22.6 $\pm$ 3.1 ^b	—	<0.001

AU = arbitrary units; NFT = neurofibrillary tangle-like structures. F values are not reported for silver optical density parameters because non-parametric Kruskal–Wallis confirmation was applied; all between-group contrasts were significant at $p < 0.001$.

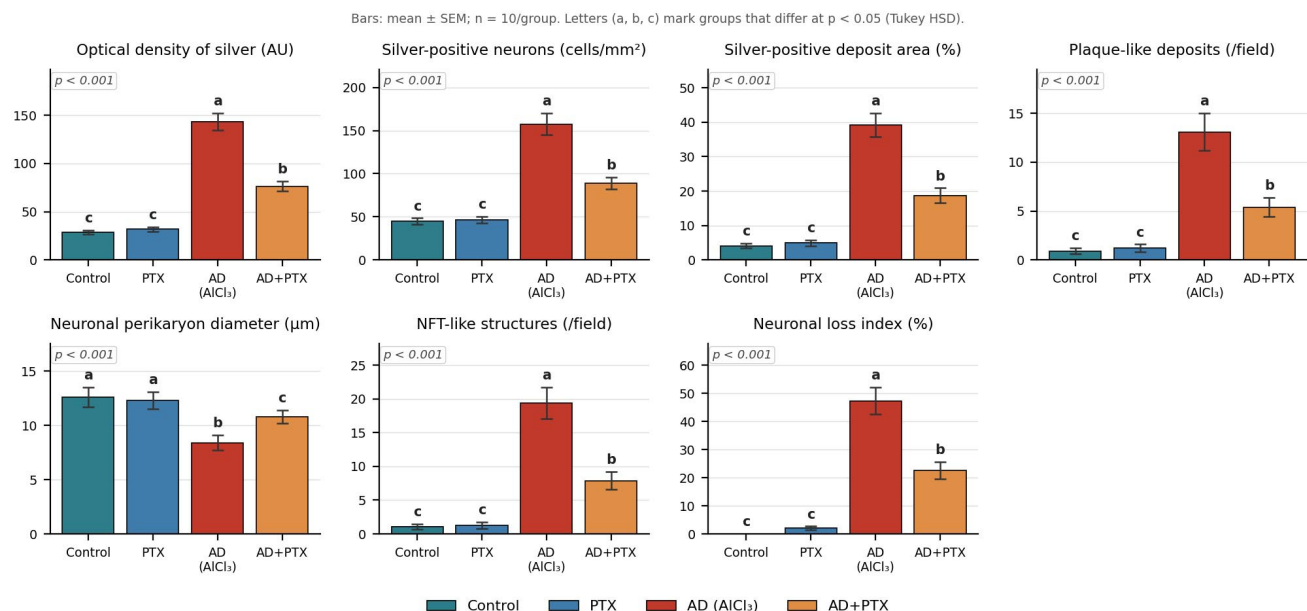


Fig. 2. Bielschowsky silver-stain morphometry (Table II). Mean $\pm$ SEM ($n = 10$); group differences confirmed by Kruskal–Wallis with Dunn's test ($p < 0.001$).

Congo Red Morphometric Analysis

Control and PTX-only sections showed only non-specific membrane staining, no extracellular CR-positive deposits, and no birefringence. AlCl₃-treated sections carried the highest amyloid burden: around 11 plaques per field, all with apple-green birefringence, and total CR-positive area increased 42-fold. Neuronal density fell about 50 % and soma area about 32 %. PTX co-treatment cut plaque count by 63 %, mean plaque area by 67 %, and total CR-positive area by 68 %, with substantial recovery of neuronal density. Every Group 4 value differed from both control and AlCl₃ ($p < 0.001$) (Table III, Fig. 3).

GFAP Immunohistochemical Morphometric Analysis

Control sections had quiescent GFAP immunoreactivity in the dentate gyrus outer molecular layer and CA1 stratum lacunosum-moleculare, with slender well-arborised astrocytes; PTX-only sections matched controls. AlCl₃-treated sections showed florid reactive astrogliosis: cell density rose 2.6-fold, cell body area doubled, and GFAP area fraction was nearly fourfold control. Reactive astrocytes had more, but shorter and thicker, processes, consistent with disease-associated astrocyte morphology (Escartin *et al.*, 2021). PTX co-treatment cut GFAP-positive cell density by 40 %, body area by 37 %, and area fraction by 43 %, with partial recovery of process length. The hypertrophy index of

1.89 placed Group 4 between controls and the AD group on every parameter (Table IV, Fig. 4).

Table III. Morphometric parameters of brain tissue sections with Congo red staining (mean $\pm$ SEM; n = 10/group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences ($p < 0.05$).

Congo Red Parameter	Control	PTX	AD (AICl ₃)	AD + PTX	F	p
Number of amyloid plaques (/field)	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	10.93 $\pm$ 0.35 ^c	4.02 $\pm$ 0.15 ^b	725.14	<0.001
Mean amyloid plaque area (μm^2)	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	2631.67 $\pm$ 69.32 ^c	868.33 $\pm$ 24.03 ^b	1143.61	<0.001
Total CR+ area per field (μm^2)	143.83 $\pm$ 2.09 ^a	146.33 $\pm$ 1.89 ^a	6088.83 $\pm$ 123.77 ^c	1928.00 $\pm$ 49.51 ^b	1768.56	<0.001
CR+ area fraction (%)	1.20 $\pm$ 0.02 ^a	1.22 $\pm$ 0.02 ^a	20.77 $\pm$ 0.46 ^c	8.82 $\pm$ 0.25 ^b	1245.95	<0.001
Neuronal density (cells/mm ²)	180.83 $\pm$ 1.42 ^a	178.33 $\pm$ 1.41 ^a	89.67 $\pm$ 2.23 ^c	139.17 $\pm$ 1.87 ^b	585.03	<0.001
Mean neuronal soma area (μm^2)	120.73 $\pm$ 0.94 ^a	119.77 $\pm$ 0.97 ^a	82.60 $\pm$ 1.00 ^c	105.73 $\pm$ 0.99 ^b	332.01	<0.001

CR+ = Congo red-positive. Apple-green birefringence under polarized light confirmed amyloid fibril identity in all CR+ deposits in Groups 3 and 4. No CR-positive plaques were detected in Groups 1 or 2.

Bars: mean $\pm$ SEM; n = 10/group. Letters (a, b, c) mark groups that differ at $p < 0.05$ (Tukey HSD).

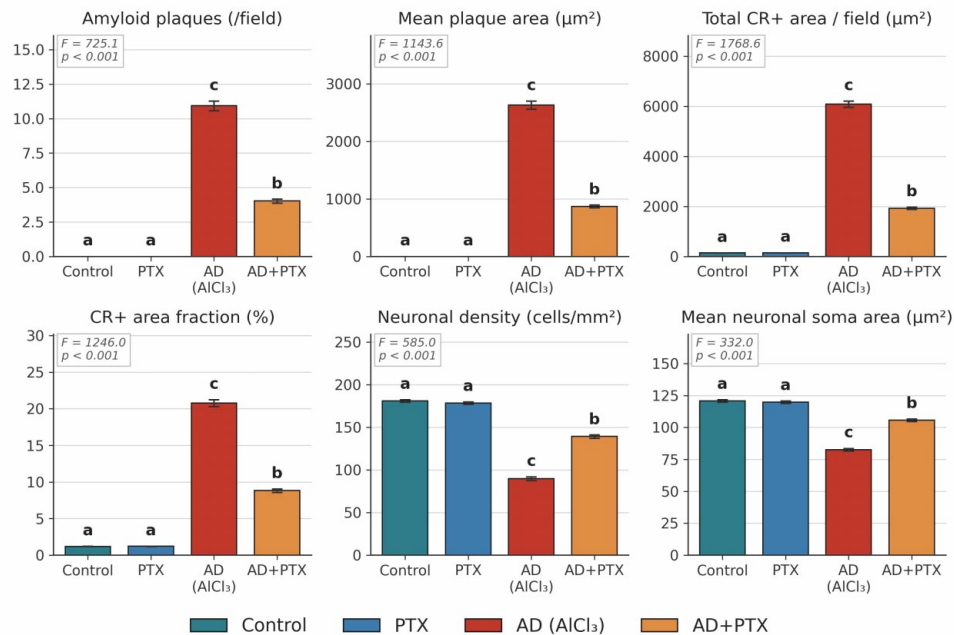


Fig. 3. Congo red amyloid morphometry under polarized light (Table III). Mean $\pm$ SEM (n = 10); letters mark groups differing at $p < 0.05$ (Tukey's HSD).

Table IV. Morphometric parameters of GFAP-positive astrocytes (mean $\pm$ SD; n = 10/group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences ($p < 0.05$).

GFAP IHC Parameter	Control	PTX	AD (AICl ₃)	AD + PTX	F	p
GF AP+ astrocyte density (cells/mm ²)	18.3 $\pm$ 2.1 ^a	20.1 $\pm$ 2.4 ^a	47.6 $\pm$ 4.8 ^c	28.4 $\pm$ 3.2 ^b	—	<0.001
Astrocyte cell body area (μm^2)	52.4 $\pm$ 5.8 ^a	55.2 $\pm$ 6.1 ^a	118.7 $\pm$ 12.4 ^c	74.3 $\pm$ 8.6 ^b	—	<0.001
Mean primary process length (μm)	31.8 $\pm$ 3.4 ^a	34.2 $\pm$ 3.7 ^a	18.6 $\pm$ 2.8 ^c	25.7 $\pm$ 3.1 ^b	—	<0.001
No. of primary processes/cell	3.8 $\pm$ 0.6 ^a	4.1 $\pm$ 0.5 ^a	6.9 $\pm$ 0.8 ^c	5.2 $\pm$ 0.7 ^b	—	<0.001
GFAP immunoreactivity area fraction (%)	9.7 $\pm$ 1.4 ^a	11.2 $\pm$ 1.6 ^a	38.4 $\pm$ 4.2 ^c	21.8 $\pm$ 3.1 ^b	—	<0.001
Amyloid plaque count (/field)	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	8.3 $\pm$ 1.2 ^c	3.1 $\pm$ 0.8 ^b	—	<0.001
Astrocyte hypertrophy index (AU)	1.00 $\pm$ 0.08 ^a	1.06 $\pm$ 0.09 ^a	3.47 $\pm$ 0.31 ^c	1.89 $\pm$ 0.22 ^b	—	<0.001

Hypertrophy index = mean cell body area in group $\div$ mean cell body area in control.

GFAP = glial fibrillary acidic protein; IHC = immunohistochemistry; $\times 400$ magnification.

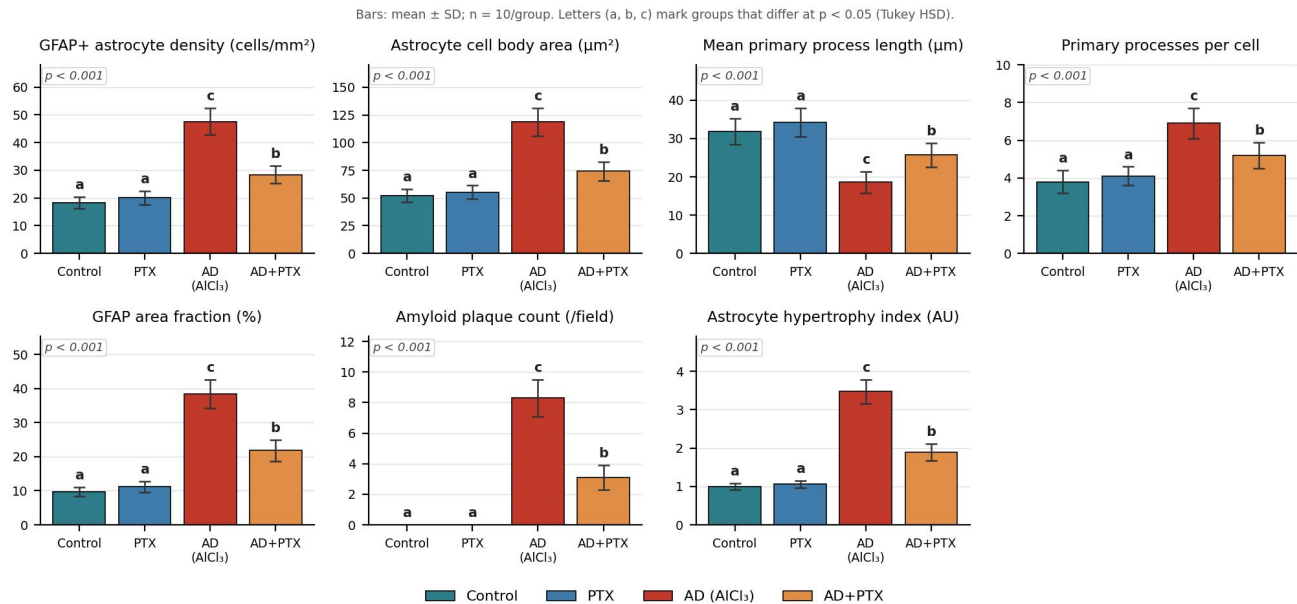


Fig. 4. GFAP immunohistochemical morphometry of reactive astrocytes (Table IV). Mean $\pm$ SD (n = 10); letters mark groups differing at $p < 0.05$ (Tukey's HSD).

Behavioral and Cognitive Assessment

Controls performed well across every paradigm. MWM escape latency fell from 52.3 ± 8.1 s on Day 1 to 15.2 ± 3.1 s on Day 5, probe-trial target quadrant time was 37.4 ± 3.8 %, and the NOR discrimination index was 0.67 ± 0.06 . PTX-only animals performed the same as controls (all $p > 0.05$). AlCl_3 produced profound cognitive impairment: the MWM learning curve was abolished: Day 5 latency (54.7 ± 7.8 s) did not differ significantly from Day 1 (73.6 ± 9.2 s, $p > 0.05$); path length was 3.4-fold control, probe-trial target

quadrant time (20.8 %) approached chance (25 %), platform crossings fell to 1.3, NOR DI collapsed to 0.11, and passive-avoidance step-through latency fell to 82.3 s. PTX co-treatment improved every behavioral parameter except Day 1 escape latency ($p < 0.001$): Day 5 escape latency dropped to 28.6 s (48 % improvement), path length 46 %, probe-trial target quadrant time recovered to 30.6 %, the NOR DI rose to 0.48 (a 4.4-fold gain), and passive-avoidance latency more than doubled to 186.5 s (Table V, Fig. 5).

Table V. Behavioral and cognitive assessment parameters (mean $\pm$ SD; n = 10/group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences ($p < 0.05$).

Behavioral Parameter	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
MWM — Escape latency Day 1 (s)	52.3 $\pm$ 8.1 ^a	50.1 $\pm$ 7.8 ^a	73.6 $\pm$ 9.2 ^b	63.4 $\pm$ 8.3 ^b	16.82	<0.001
MWM — Escape latency Day 3 (s)	31.4 $\pm$ 5.2 ^a	29.8 $\pm$ 4.9 ^a	67.8 $\pm$ 8.7 ^c	48.2 $\pm$ 7.1 ^b	71.34	<0.001
MWM — Escape latency Day 5 (s)	15.2 $\pm$ 3.1 ^a	14.8 $\pm$ 2.9 ^a	54.7 $\pm$ 7.8 ^c	28.6 $\pm$ 5.4 ^b	148.71	<0.001
MWM — Path length Day 5 (cm)	142.3 $\pm$ 18.4 ^a	138.6 $\pm$ 16.2 ^a	487.2 $\pm$ 62.4 ^c	264.8 $\pm$ 38.7 ^b	183.56	<0.001
MWM probe — Target quadrant time (%)	37.4 $\pm$ 3.8 ^a	38.1 $\pm$ 3.4 ^a	20.8 $\pm$ 3.2 ^c	30.6 $\pm$ 3.7 ^b	64.23	<0.001
MWM probe — Platform crossings (n)	4.8 $\pm$ 0.9 ^a	5.1 $\pm$ 0.8 ^a	1.3 $\pm$ 0.5 ^c	3.1 $\pm$ 0.7 ^b	73.18	<0.001
NOR — Discrimination index (DI)	0.67 $\pm$ 0.06 ^a	0.69 $\pm$ 0.05 ^a	0.11 $\pm$ 0.04 ^c	0.48 $\pm$ 0.06 ^b	249.87	<0.001
NOR — Recognition index (RI)	0.83 $\pm$ 0.03 ^a	0.84 $\pm$ 0.03 ^a	0.56 $\pm$ 0.04 ^c	0.74 $\pm$ 0.04 ^b	98.46	<0.001
Passive avoidance — Step-through latency (s)	268.4 $\pm$ 21.6 ^a	271.8 $\pm$ 18.4 ^a	82.3 $\pm$ 14.7 ^c	186.5 $\pm$ 22.8 ^b	156.23	<0.001

Bars: mean $\pm$ SD; n = 10/group. Letters (a, b, c) mark groups that differ at $p < 0.05$ (Tukey HSD).

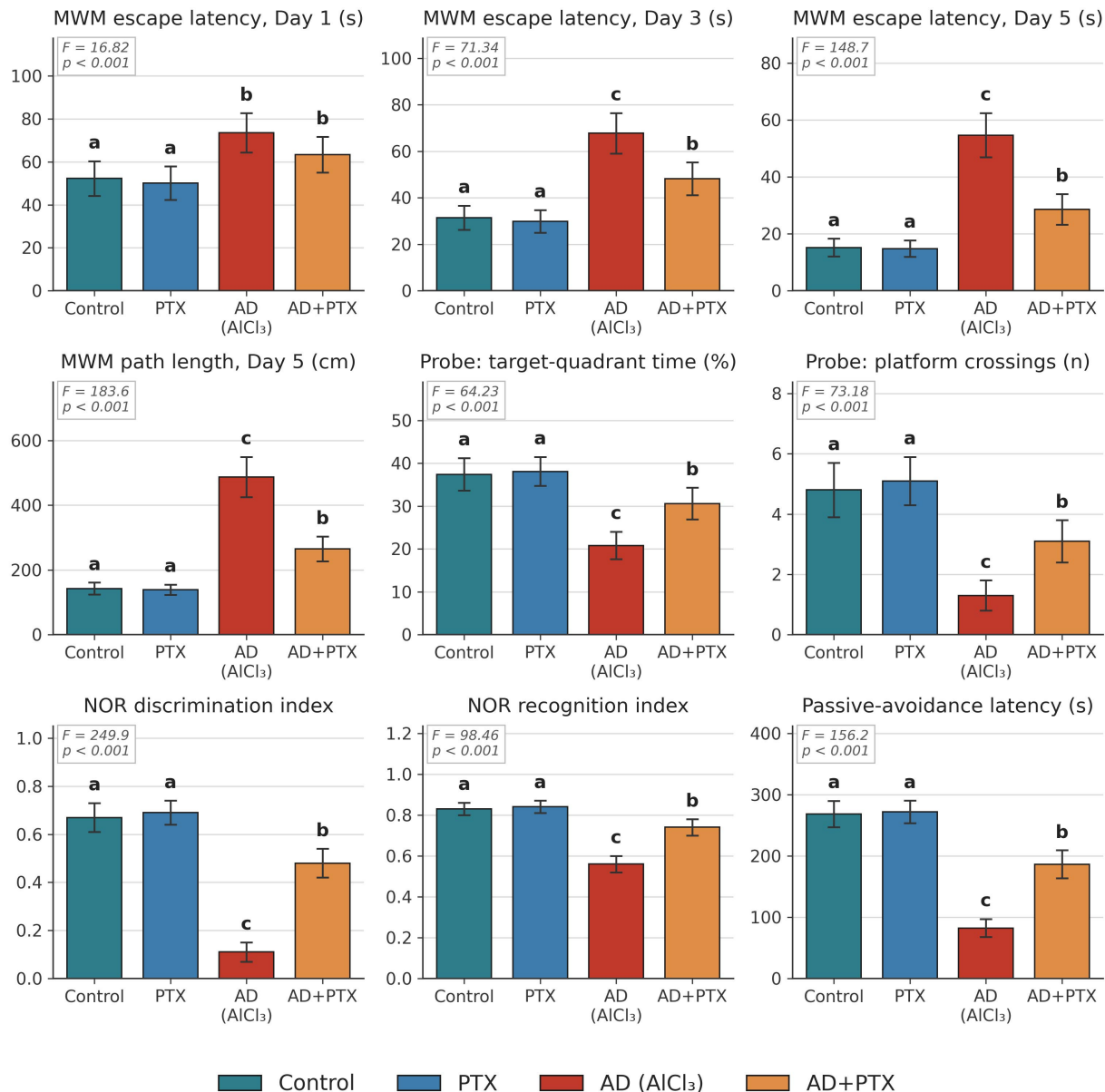


Fig. 5. Behavioral and cognitive outcomes (Table V): Morris water maze, novel object recognition, and passive avoidance. Mean $\pm$ SD (n = 10); letters mark groups differing at $p < 0.05$ (Tukey's HSD).

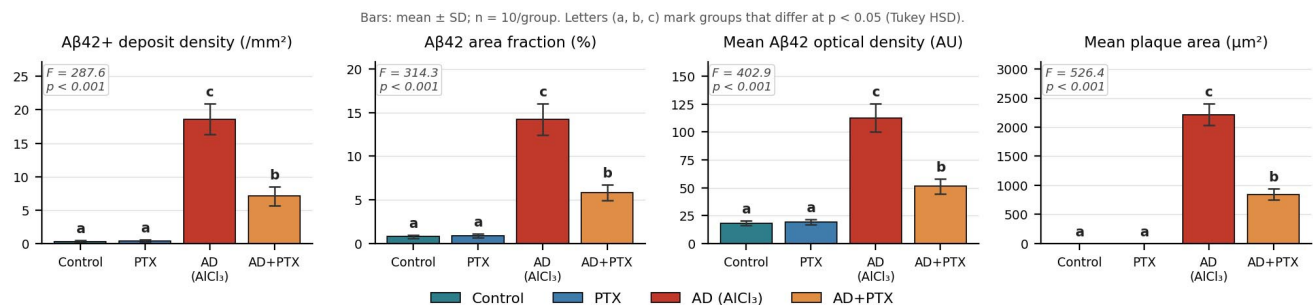
A β 42 and Phospho-tau (AT8) Immunohistochemical Analysis

Control and PTX-only sections showed only sparse A β 42 immunoreactivity (occasional pericapillary staining) and rare CA3 perikaryal AT8 immunoreactivity, consistent with basal physiological tau phosphorylation (Braak *et al.*, 2006). AlCl₃-treated sections had heavy A β 42 immunoreactivity throughout CA1, CA3, and dentate gyrus

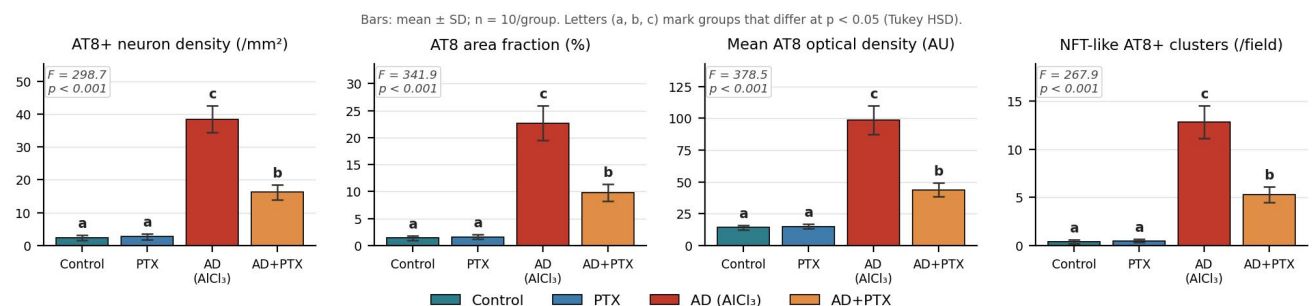
(deposit density 18.6/mm²; optical density 6.1-fold control), with a 16.7-fold rise in AT8-positive neuron density. A β 42 deposits co-localized spatially with Congo red-positive plaques on parallel sections, confirming amyloid identity. PTX cut A β 42 burden by 54–62 % across parameters and AT8 by 56–59 %, in line with the 58.8–68.3 % drop in Congo red amyloid burden and the 59.3 % drop in Bielschowsky NFT-like structures from the same animals (Table VI, Fig. 6; Table VII, Fig. 7).

Table VI. Morphometric parameters of A β 42 immunohistochemistry (mean $\pm$ SD; n = 10/group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences (p < 0.05).

A β 42 IHC Parameter	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
A β 42-positive deposit density (/mm ²)	0.3 $\pm$ 0.2 ^a	0.4 $\pm$ 0.2 ^a	18.6 $\pm$ 2.3 ^c	7.1 $\pm$ 1.4 ^b	287.64	<0.001
A β 42 immunoreactive area fraction (%)	0.8 $\pm$ 0.2 ^a	0.9 $\pm$ 0.2 ^a	14.2 $\pm$ 1.8 ^c	5.8 $\pm$ 0.9 ^b	314.29	<0.001
Mean A β 42 optical density (AU)	18.4 $\pm$ 2.1 ^a	19.2 $\pm$ 2.3 ^a	112.7 $\pm$ 12.6 ^c	51.4 $\pm$ 6.8 ^b	402.87	<0.001
Mean plaque area (μ m ²)	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	2218.4 $\pm$ 183.6 ^c	843.7 $\pm$ 94.2 ^b	526.41	<0.001

Fig. 6. A β 42 immunohistochemical morphometry (Table VI). Mean $\pm$ SD (n = 10); letters mark groups differing at p < 0.05 (Tukey's HSD).Table VII. Morphometric parameters of phospho-tau (AT8) immunohistochemistry (mean $\pm$ SD; n = 10/group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences (p < 0.05). AT8 recognizes tau phosphorylated at Ser202/Thr205.

AT8 Phospho-tau Parameter	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
AT8-positive neuron density (/mm ²)	2.3 $\pm$ 0.8 ^a	2.7 $\pm$ 0.9 ^a	38.4 $\pm$ 4.1 ^c	16.2 $\pm$ 2.3 ^b	298.73	<0.001
AT8 immunoreactive area fraction (%)	1.4 $\pm$ 0.4 ^a	1.6 $\pm$ 0.4 ^a	22.7 $\pm$ 3.2 ^c	9.8 $\pm$ 1.6 ^b	341.86	<0.001
Mean AT8 optical density (AU)	14.2 $\pm$ 1.8 ^a	15.1 $\pm$ 2.0 ^a	98.6 $\pm$ 11.3 ^c	43.7 $\pm$ 5.4 ^b	378.54	<0.001
NFT-like AT8+ neurite clusters (/field)	0.4 $\pm$ 0.2 ^a	0.5 $\pm$ 0.2 ^a	12.8 $\pm$ 1.7 ^c	5.3 $\pm$ 0.8 ^b	267.93	<0.001

Fig. 7. Phospho-tau (AT8) immunohistochemical morphometry (Table VII). Mean $\pm$ SD (n = 10); letters mark groups differing at p < 0.05 (Tukey's HSD).

Biochemical Profile of Hippocampal Tissue

Control and PTX-only animals had essentially identical biochemical profiles, so PTX on its own did not alter baseline neurochemistry, antioxidant defenses, or low-grade inflammatory tone. AlCl₃ exposure produced a full biochemical signature of Alzheimer-type pathology: tissue

A β 42 rose 4.6-fold, the cholinergic markers shifted sharply (ACh fell to 23 % of control, AChE rose about 4-fold), TNF- α , IL-6, and ICAM-1 increased 1.9-, 4.1-, and 4.1-fold respectively, GSH and SOD each dropped about 29 %, and MDA rose about 20 %.

PTX co-treatment moved every biochemical parameter significantly toward normal ($p < 0.001$ for all primary contrasts). Tissue A β 42 fell 43 % relative to AD; ICAM-1 by 59 %; IL-6 by 60 %; and AChE activity by 29 %, with a 2.4-fold rise in acetylcholine. Two markers (TNF- α

and MDA) returned to the control range, sharing letter “a” with controls in the Tukey HSD analysis. The remaining parameters showed substantial but incomplete recovery, consistent with the partial neuroprotection seen on histology (Table VIII, Fig. 8).

Bars: mean $\pm$ SD; $n = 10$ /group. Letters (a, b, c) mark groups that differ at $p < 0.05$ (Tukey HSD).

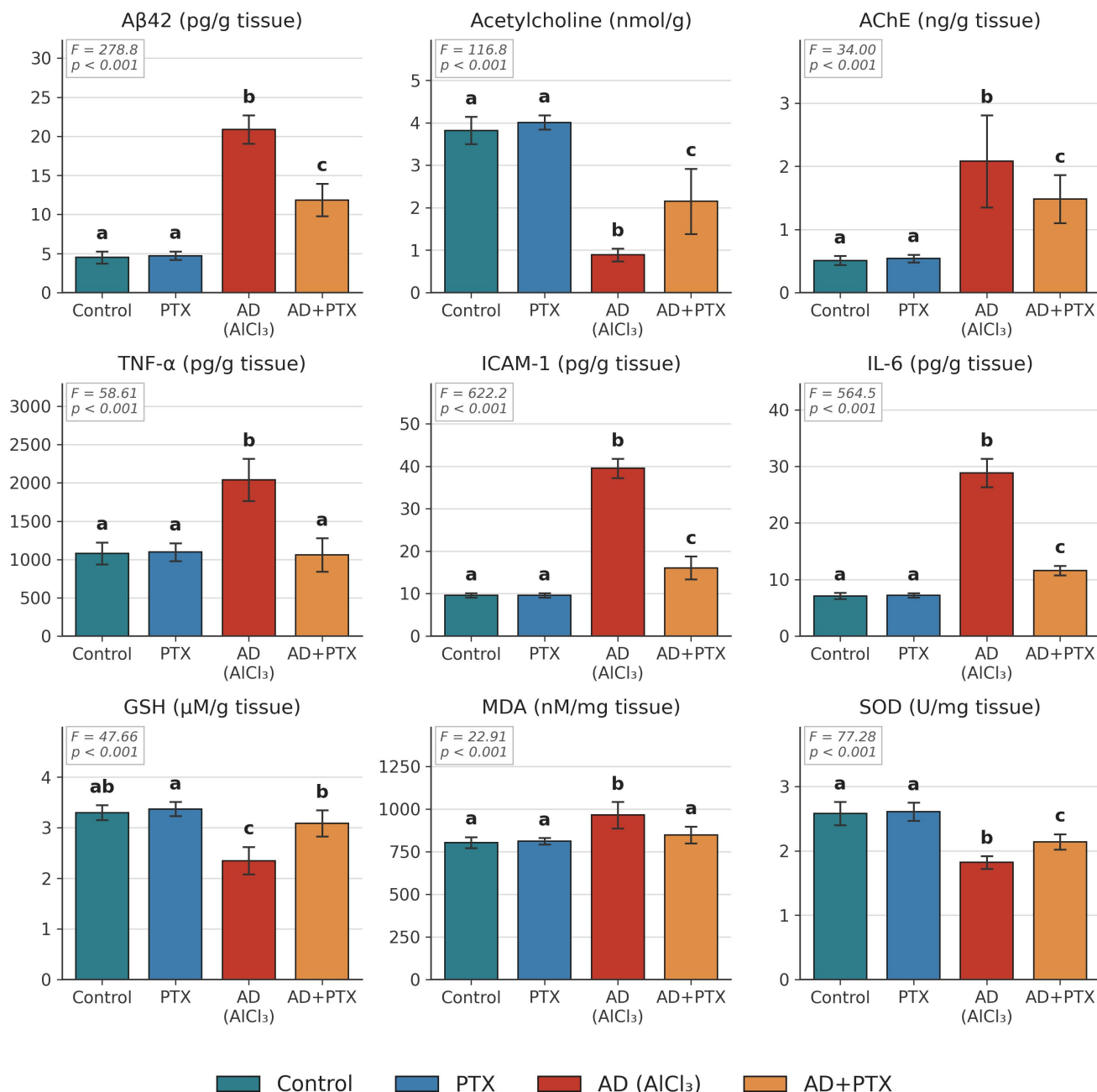


Fig. 8. Hippocampal biochemical parameters (Table VIII): amyloid, cholinergic, inflammatory, and oxidative-stress markers. Mean $\pm$ SD ($n = 10$); letters mark groups differing at $p < 0.05$ (Tukey's HSD).

Table VIII. Hippocampal biochemical parameters (mean $\pm$ SD; n = 10/group; one-way ANOVA + Tukey HSD). Different superscripts indicate significant between-group differences ($p < 0.05$); groups sharing a letter were not significantly different.

Parameter (Unit)	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
A β 42 (pg/g tissue)	4.50 $\pm$ 0.75 ^a	4.72 $\pm$ 0.54 ^a	20.88 $\pm$ 1.83 ^b	11.86 $\pm$ 2.09 ^c	278.76	<0.001
Acetylcholine (nmol/g tissue)	3.82 $\pm$ 0.32 ^a	4.01 $\pm$ 0.17 ^a	0.89 $\pm$ 0.15 ^b	2.15 $\pm$ 0.77 ^c	116.83	<0.001
AChE (ng/g tissue)	0.51 $\pm$ 0.07 ^a	0.54 $\pm$ 0.06 ^a	2.08 $\pm$ 0.73 ^b	1.48 $\pm$ 0.38 ^c	34.00	<0.001
TNF- α (pg/g tissue)	1079.0 $\pm$ 143.1 ^a	1096.3 $\pm$ 117.7 ^a	2041.6 $\pm$ 276.6 ^b	1059.4 $\pm$ 218.3 ^a	58.61	<0.001
ICAM-1 (pg/g tissue)	9.63 $\pm$ 0.52 ^a	9.63 $\pm$ 0.52 ^a	39.53 $\pm$ 2.28 ^b	16.06 $\pm$ 2.69 ^c	622.18	<0.001
IL-6 (pg/g tissue)	7.09 $\pm$ 0.55 ^a	7.20 $\pm$ 0.41 ^a	28.85 $\pm$ 2.52 ^b	11.58 $\pm$ 0.84 ^c	564.51	<0.001
GSH (μ M/g tissue)	3.30 $\pm$ 0.15 ^{ab}	3.37 $\pm$ 0.14 ^a	2.35 $\pm$ 0.27 ^c	3.09 $\pm$ 0.26 ^b	47.66	<0.001
MDA (nM/mg tissue)	802.5 $\pm$ 32.5 ^a	811.1 $\pm$ 18.5 ^a	963.9 $\pm$ 76.9 ^b	847.5 $\pm$ 48.4 ^a	22.91	<0.001
SOD (U/mg tissue)	2.58 $\pm$ 0.18 ^a	2.61 $\pm$ 0.14 ^a	1.82 $\pm$ 0.10 ^b	2.14 $\pm$ 0.12 ^c	77.28	<0.001

A β 42 = amyloid- β 42; AChE = acetylcholinesterase; ICAM-1 = intercellular adhesion molecule-1; GSH = reduced glutathione; MDA = malondialdehyde; SOD = superoxide dismutase. F-statistics and exact p-values are from one-way ANOVA.

Hippocampal BAX and Bcl-2 Gene Expression

Bcl-2 expression matched between control and PTX-only animals (16.5 vs 15.9), so PTX on its own does not alter baseline pro-survival signaling. AlCl₃ exposure produced a polarized shift: Bcl-2 transcript dropped to 60 % of control (9.9), BAX rose 28 % (35.2), and the BAX/Bcl-2 ratio more than doubled (1.67 $\rightarrow$ 3.56). PTX co-treatment

moved the balance back: Bcl-2 recovered to 13.7 (38 % above AD), BAX fell to 29.6 (16 % below AD), and the BAX/Bcl-2 ratio dropped to 2.16, about 39 % of the way back to the control set-point. Both genes stayed statistically distinct from control (letter “b”), consistent with partial rather than full restoration of apoptotic balance, and matching the histological recovery of neuronal density and the parallel drop in pyknotic neurons in Group 4 (Table IX, Fig. 9).

Table IX. Relative hippocampal expression of the pro-apoptotic gene BAX and the anti-apoptotic gene Bcl-2 by qRT-PCR (mean $\pm$ SD; n = 10/group; one-way ANOVA + Tukey HSD). The BAX/Bcl-2 ratio is a derived index of apoptotic balance, with higher values indicating greater pro-apoptotic drive.

Gene / Index	Control	PTX	AD (AlCl ₃)	AD + PTX	F	p
Bcl-2 (relative expression)	16.48 $\pm$ 2.02 ^a	15.88 $\pm$ 0.84 ^a	9.90 $\pm$ 0.48 ^c	13.70 $\pm$ 0.89 ^b	61.16	<0.001
BAX (relative expression)	27.60 $\pm$ 0.61 ^a	27.96 $\pm$ 0.85 ^a	35.20 $\pm$ 0.99 ^c	29.62 $\pm$ 1.14 ^b	146.71	<0.001
BAX/Bcl-2 ratio (derived)	1.67 $\pm$ 0.21 ^a	1.76 $\pm$ 0.10 ^a	3.56 $\pm$ 0.20 ^c	2.16 $\pm$ 0.16 ^b	—	<0.001

Expression values were calculated by the comparative Ct ($2^{-\Delta\Delta Ct}$) method after normalization to an internal housekeeping gene. Triplicate technical replicates were averaged per animal before group-level analysis.

Bars: mean $\pm$ SD; n = 10/group. Letters (a, b, c) mark groups that differ at $p < 0.05$ (Tukey HSD).

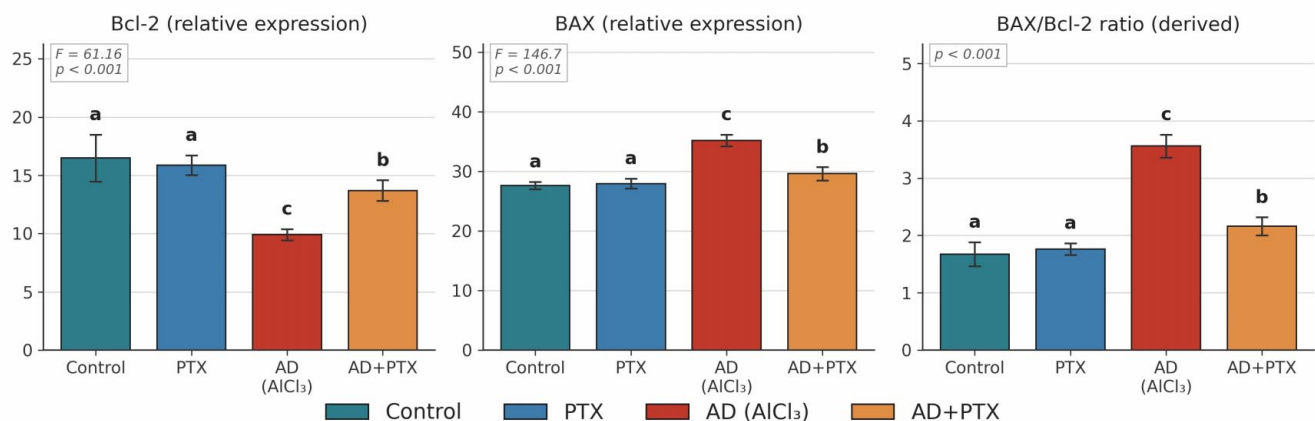


Fig. 9. Hippocampal BAX and Bcl-2 expression and the derived BAX/Bcl-2 ratio (Table IX). Mean $\pm$ SD (n = 10); letters mark groups differing at $p < 0.05$ (Tukey's HSD).

DISCUSSION

This study examined pentoxifylline's neuroprotective effect in the AlCl_3 model of Alzheimer's disease, drawing histology, immunohistochemistry, biochemistry, and gene expression from the same animals. The readouts tell a consistent story: AlCl_3 produces severe, multi-dimensional hippocampal neurodegeneration; pentoxifylline blunts much of the damage without fully reversing it; and the breadth of its effect points to action across several linked pathways rather than a single one.

The AlCl_3 model

The histological findings agree with one another. H&E showed neuronal loss, pyknosis, and spongiosis. Silver staining confirmed neurofibrillary tangle-like pathology and argentophilic plaques. Congo red under polarized light gave a definitive read on amyloid through apple-green birefringence — the criterion that separates true amyloid from non-specific Congo red binding, and one that rodent AD characterizations often skip. GFAP immunohistochemistry showed marked reactive astrogliosis with the morphology of disease-associated astrocytes (Escartin *et al.*, 2021). The biochemistry pointed the same way: tissue $\text{A}\beta_{42}$ up 4.6-fold, cholinergic signaling collapsed, IL-6 and ICAM-1 up nearly fourfold, TNF- α doubled, and a coordinated slide toward oxidative stress. None of this means the model captures the genetic, progressive, or age-dependent character of human AD; it is a neurotoxicological model that reproduces a selected set of endpoints, and the translation has to be read with that limit in mind.

Aluminum drives this picture by disrupting several converging pathways at once. It pushes APP down the amyloidogenic route by upregulating BACE1, seeds and accelerates $\text{A}\beta$ fibrillization, hampers $\text{A}\beta$ clearance through lysosomal and glymphatic dysfunction, and activates GSK-3 β and CDK5, the kinases behind pathological tau hyperphosphorylation (Walton, 2014; Llorens-Martín *et al.*, 2014; Islam *et al.*, 2022). The resulting amyloid and tau load activates microglia and astrocytes, and the inflammatory milieu that follows accelerates the neuronal loss seen across our H&E and Congo red data and the rise in the BAX/Bcl-2 ratio in Table IX.

PTX's multi-target profile

PTX improved every endpoint we measured. A single mechanism struggles to explain that breadth, which instead suggests PTX is acting on several of the pathways aluminum disrupts.

Anti-inflammatory action

The biggest single-parameter improvements were in the inflammatory readouts: ICAM-1 fell 59 %, IL-6 by 60 %, and TNF- α returned to baseline. These results fit PTX's reported suppression of NF- κ B-driven neuroinflammatory gene expression in other models (Neves *et al.*, 2015; Vakili *et al.*, 2011) and align with the drug interrupting the neuroinflammatory amplification loop that accelerates amyloid and tau pathology in this model (Neves *et al.*, 2015), though direct confirmation here would require Western blotting or NF- κ B activity assays.

cAMP/PKA/CREB and the BAX/Bcl-2 axis

PTX inhibits PDE4 and PDE5, raises intracellular cAMP, activates PKA, and promotes CREB phosphorylation. CREB drives expression of BDNF, Bcl-2, and other pro-survival genes (Khalifa *et al.*, 2022; Wang *et al.*, 2020). Our gene-expression data fit this mechanism: AlCl_3 produced a more-than-twofold rise in the BAX/Bcl-2 ratio, and PTX moved the ratio back toward control by 39 %, with both genes moving in opposite directions (Bcl-2 up, BAX down). The partial recovery of neuronal density and soma area across all three histological stains fits this rebalancing: neurons on the edge of apoptotic commitment could be salvaged by CREB-dependent survival signals; those past the threshold could not.

Anti-amyloidogenic and tau-modulating activity

The 58.8–67.0 % reductions in silver-stain plaque-like deposits and Congo red-confirmed plaque number and area, together with the 43 % drop in tissue $\text{A}\beta_{42}$ measured biochemically, suggest PTX influences $\text{A}\beta$ production or clearance, though the specific mechanism was not directly tested here. Candidate mechanisms from the literature include cAMP-mediated neprilysin upregulation (Khalifa *et al.*, 2022) and NF- κ B-associated BACE1 modulation (Walton, 2014), both of which need direct confirmation. The 59 % drop in silver-stain NFT-like structures, matched by the 56–59 % drop in AT8 immunoreactivity, points to attenuation of tau hyperphosphorylation at Ser202/Thr205; cAMP-mediated GSK-3 β inhibition is a plausible candidate (Khalifa *et al.*, 2022).

Cholinergic preservation

AlCl_3 caused a dramatic cholinergic collapse: acetylcholine fell to 23 % of control and AChE rose fourfold, biochemically reproducing the cholinergic deficit of clinical AD. PTX co-treatment more than doubled ACh and cut AChE activity by about 29 %. PTX is not itself a

cholinesterase inhibitor, so this cholinergic recovery is most plausibly a downstream consequence of preserved cholinergic neurons (higher H&E neuronal density), lower neuroinflammatory pressure, and reduced apoptotic drive.

Antioxidant and haemorheological mechanisms

Aluminum generates reactive oxygen species through Fenton-type reactions, driving lipid peroxidation and mitochondrial dysfunction. PTX reduces MDA, restores SOD and catalase activity, and inhibits lipid peroxidation in aluminum-exposed brain tissue (Neves *et al.*, 2015; Wang *et al.*, 2020). Our biochemistry reproduces this pattern: MDA returned to control values; SOD and GSH recovered substantially without fully normalizing. PTX also increases erythrocyte deformability, reduces plasma viscosity, and inhibits platelet aggregation, improving cerebral microvascular perfusion (Vakili *et al.*, 2011; Nedergaard & Goldman, 2020), a vascular mechanism that may help amyloid clearance through better perivascular glymphatic drainage (Nedergaard & Goldman, 2020).

The significance of GFAP-confirmed reactive astrogliosis

Reactive astrogliosis is often dismissed as a bystander in AD, but the evidence increasingly casts it as an active driver. The 3.47-fold rise in the astrocyte hypertrophy index and the near-fourfold rise in GFAP area fraction in Group 3, together with the shift from quiescent astrocytes (long, thin, well-arborized) to reactive ones (shorter, thicker, more numerous), are the signature of disease-associated reactive astrogliosis described by Escartin *et al.* (2021) and Sofroniew (2020) — a state that amplifies neuroinflammation, unbalances glutamate handling, weakens potassium buffering, and starves neurons of metabolic support.

The 40 % reduction in GFAP-positive cell density and the 43 % drop in GFAP area fraction with PTX therefore reflect attenuation of an active disease-propagating mechanism. PTX's restoration of astrocyte process length (from 18.6 to 25.7 μm) is the most informative single change here, because the shift from reactive to quiescent astrocyte morphology is associated with recovery of homeostatic astrocyte functions, including glutamate uptake via EAAT2, potassium buffering via Kir4.1, and lactate provision via monocarboxylate transporters (Sofroniew, 2020).

Cross-platform consistency

The treatment effects agree across platforms. Amyloid reductions ran from 43 % (tissue A β 42 by ELISA) to 68 % (Congo red total CR-positive area), with A β 42 IHC area fraction (-59 %) sitting between them. Astrocytic and

inflammatory markers fell by 40 % (GFAP cell density) to 60 % (IL-6). Cognitive recovery landed in the same band (46–48 % on the MWM, a 4.4-fold gain on NOR DI). Gene expression added a further layer: a 39 % normalization of the BAX/Bcl-2 ratio that tracks the 51 % recovery of neuronal density. That a stain, an antibody, an ELISA, and a transcript all point to similar effect sizes argues we are seeing real biology rather than an assay-specific artefact. The residual gap between Group 4 and control is just as consistent, fitting the reading of substantial but incomplete neuroprotection under a concurrent design.

The cholinergic deficit and its reversal

One of the more clinically resonant findings here is the biochemical reproduction of the cholinergic deficit that defines symptomatic AD. ACh dropped to about 23 % of control alongside a fourfold rise in AChE activity, mirroring the cholinergic collapse that motivated cholinesterase-inhibitor pharmacology. The PTX-treated cohort more than doubled ACh and reduced AChE activity substantially, with both still distinct from controls. Since PTX is not itself a cholinesterase inhibitor, the cholinergic restoration here is most plausibly a downstream consequence of upstream protection: preserved cholinergic neurons (H&E neuronal density), reduced neuroinflammatory pressure (lower TNF- α , IL-6, ICAM-1), and reduced apoptotic drive (corrected BAX/Bcl-2 ratio).

Behavioral validation

The behavioral tests ask whether the histology corresponds to real functional loss and real rescue. The Morris water maze shows that AlCl₃-induced hippocampal degeneration produces a measurable spatial-memory deficit: Group 3 essentially failed to learn (Day 5 latency 54.7 $\pm$ 7.8 s, vs 73.6 $\pm$ 9.2 s, on Day 1, $p > 0.05$), indicating that CA1 neuronal loss crossed the threshold for functional decompensation of the hippocampo-entorhinal navigation circuit (Morris, 1984; Liaquat *et al.*, 2019). The NOR deficit (DI = 0.11) points to concurrent perirhinal and hippocampal disruption, matching the histology, and passive avoidance gave a third independent readout: AlCl₃ animals re-entered the dark compartment after about 82 s, against roughly 270 s in controls — a failure of aversive memory consolidation.

PTX co-treatment produced substantial cognitive rescue across every behavioral measure. The 48 % improvement in Day 5 escape latency and 46 % reduction in path length show partial but meaningful restoration of spatial learning, and the recovery of probe-trial target quadrant time to 30.6 % (*versus* chance at 25 %) confirms re-establishment of consolidated spatial memory. The

passive-avoidance latency more than doubled to 187 s. The behavioral improvement (45–48 % across MWM measures) lines up quantitatively with the histological neuroprotection (58–68 % drop in amyloid burden, 56–59 % drop in AT8 phospho-tau, 50–60 % drop in neuroinflammatory markers). The recovery of NOR DI to 0.48, a 4.4-fold gain, is particularly meaningful because NOR is more sensitive to early-stage hippocampal dysfunction than MWM escape latency (Ennaceur & Delacour, 1988).

Apoptotic balance

The qRT-PCR data on BAX and Bcl-2 add a molecular dimension to the widespread neuronal pyknosis and loss seen on histology. The BAX/Bcl-2 ratio is one of the more robust molecular indicators of apoptotic commitment: higher values drive cytochrome c release, caspase-9 activation, and the intrinsic apoptotic pathway. The more-than-doubling of this ratio in Group 3, and its 39 % correction by PTX, fit the neuronal-density data and point to a genuine shift in the cellular survival–death balance rather than a purely descriptive change. The ratio in AlCl_3 animals ($1.67 \rightarrow 3.56$) tracks the 64 % drop in neuronal density and the 72 % pyknotic index documented histologically. PTX co-treatment cut the ratio by 39 %, mainly by recovering Bcl-2 ($9.9 \rightarrow 13.7$) and partly by reducing BAX ($35.2 \rightarrow 29.6$). The direction and magnitude match what PTX's documented cAMP/PKA/CREB activity would predict, since CREB upregulates Bcl-2 via CRE elements in its promoter. Protein-level BAX/Bcl-2, cytochrome c release, and caspase activity were not measured here; the gene-expression direction supports apoptotic regulation as a candidate mechanism worth direct mechanistic follow-up.

Limits of the current study

Several limitations deserve a plain statement. First, ten animals per group gives solid power for the primary between-group comparisons, but the ten-per-group histological subset is underpowered for subgroup analysis; future work should increase n across all platforms. Second, we did not confirm the molecular pathways directly (no Western blots for NF- κ B p65, BACE1, neprilysin, or GSK-3 β , and no kinase or phospho-protein assays), so the mechanistic discussion rests on published work read alongside our gene-expression data. Third, the study used male rats only — a real constraint, given the sex differences in AD prevalence and neuroinflammatory response. Fourth, the concurrent design tests neuroprotection, not reversal of established disease. Fifth, we did not show A β 42 and phospho-tau in the same plaque, because the two stains came from separate sections; double-labelling confocal immunofluorescence (Thioflavin-S with AT8) would settle

that. Sixth, BAX and Bcl-2 were measured only as transcripts, so protein-level confirmation, ideally with cell-type resolution, would strengthen the apoptotic-balance reading.

CONCLUSION

Chronic intraperitoneal AlCl_3 (100 mg/kg/day for 60 days) produced verified AD-type hippocampal pathology across histology, immunohistochemistry, biochemistry, behavior, and gene expression. Given concurrently, pentoxifylline (10 mg/kg/day, i.p.) cut amyloid burden by 43–68 %, phospho-tau by 56–59 %, and cytokines by 50–60 % (with TNF- α and MDA back to control), and reduced astrogliosis by 40–43 %. It corrected the BAX/Bcl-2 ratio by 39 %, recovered more than half of the cognitive deficit across three behavioral tests, and improved every biochemical marker. The mechanistic accounts here are literature-supported hypotheses awaiting direct molecular confirmation. Because the design tests prevention rather than treatment, delayed-treatment studies in both sexes, with a dose–response, are the necessary next step before any move toward the clinic.

HEGAZY, G. A.; MUSTAFA, H.N. & EL AWDAN, S. A. Posibles efectos beneficiosos de la pentoxifilina contra la enfermedad de Alzheimer inducida en un modelo de rata. *Int. J. Morphol.*, 44(4):1182-1197, 2026.

RESUMEN: La enfermedad de Alzheimer es la causa más común de demencia, y actualmente no existe ninguna terapia que revierta la neurodegeneración subyacente. En ratas, la administración crónica de cloruro de aluminio (AlCl_3) reproduce las lesiones principales de la enfermedad: depósito de amiloide- β , hiperfosforilación de tau, astrogliosis reactiva y pérdida neuronal en el hipocampo. La pentoxifilina (PTX) es un inhibidor oral de la fosfodiesterasa con acciones antiinflamatorias, antioxidantes y hemorreológicas, lo que la convierte en una posible candidata para su reutilización. Cuarenta ratas Wistar macho se dividieron en cuatro grupos iguales ($n = 10$): vehículo, PTX, AlCl_3 y AlCl_3 + PTX. Se administró AlCl_3 (100 mg/kg/día, i.p.) y PTX (10 mg/kg/día, i.p.) durante 60 días. Evaluamos la histología e inmunohistoquímica (A β 42, AT8, GFAP), paneles ELISA del hipocampo y qRT-PCR para BAX y Bcl-2, y comparamos los grupos mediante ANOVA de una vía con HSD de Tukey. El AlCl_3 produjo patología hipocámpal grave. La densidad neuronal disminuyó en un 64 %, el índice picnótico alcanzó el 72 %, el rojo Congo reveló alrededor de 11 placas amiloides birrefringentes por campo, y la fracción de área de GFAP aumentó casi cuatro veces; TNF- α , IL-6 e ICAM-1 aumentaron de 1,9 a 4,1 veces. El péptido beta-amiloide 42 tisular aumentó 4,6 veces, la acetilcolina disminuyó al 23 % del control y la relación BAX/Bcl-2 se duplicó con creces. El tratamiento con PTX mejoró significativamente todos los parámetros ($p < 0,001$): la carga amiloide se redujo entre un 58 % y un 68 %, la fosfo-tau entre un 56 % y un 59 %, la astrogliosis entre un 40 % y un 43 %, y los déficits cognitivos

entre un 46 % y un 48 %. El TNF- α y el MDA volvieron a los valores de control. El PTX produjo una neuroprotección sustancial, aunque parcial. El patrón se ajusta a la farmacología conocida del fármaco, pero el estudio genera hipótesis en lugar de confirmarlas: se utilizaron únicamente ratas macho, un esquema de tratamiento concurrente en lugar de diferido y una dosis única. Se necesitan estudios de tratamiento diferido, en ambos sexos y de dosis-respuesta antes de cualquier paso clínico.

PALABRAS CLAVE: Enfermedad de Alzheimer; Péptidos beta-amiloides; Proteínas tau; Gliosis; Estrés oxidativo; Pentoxifilina.

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