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C3H mouse model of Alzheimer's disease: Blood markers, proteomic biomarkers, cognitive ability, and histopathology

Dinda Aliffia[1], Hevi Wihadmadyatami[1*], Muhammad Z. Z. Raihan[1], Ulayatul Kustiati[2], Wilda B. T. Sanjaya[1], Anisa P. Aviana[1], Dwi A. A. Nugrahaningsih[3], Wahyu Tri Widayati[4], Srikanth Karnati[5] and Dwi Liliek Kusindarta[1]

[1]*Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[2]*Department of Pharmacology, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia*
[3]*Department of Pharmacology and Therapy, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[4]*Regional General Hospital of Karanganyar Regency, Karanganyar, Indonesia*
[5]*Institute of Anatomy and Cell Biology, Julius Maximillian University, Wuerzburg, Germany*

ABSTRACT

Background: Alzheimer's disease (AD) is a neurodegenerative condition, and the number of cases of AD is projected to increase each year. Developing an AD animal model has a major impact on studying the pathology of the disease and on developing therapies and treatments.

Aim: This study aimed to create an AD animal model using C3H mice by administering trimethyltin (TMT) via intraperitoneal injection. Hematological analysis, pathology, protein biomarkers, and behavioral assessments supported the findings.

Methods: In this experiment, two groups were included: a non-treated group (normal mouse) and a treatment group (AD animal model). Each group consisted of four male C3H mice aged 8 weeks. The treatment group was intraperitoneally injected with 2.5 mg/kg of body weight TMT. Hematological analyses were conducted to assess the blood routine, while pathological changes in brain structure, particularly in the hippocampus, were examined using hematoxylin and eosin staining as well as Nissl staining. Additionally, proteomic profiling was used to analyze protein biomarkers associated with AD via liquid chromatography-high-resolution mass spectrometry. Behavioral analysis was conducted using the radial arm maze.

Results: Hematological analysis revealed an increase in hematocrit, mean corpuscular volume, leucocytes, and neutrophil levels, whereas other parameters remained within the normal range. Histopathological analysis revealed neuronal loss and structural alterations in the pyramidal cell layers of the CA1 and CA3, the presence of inflammation, and neurofibrillary tangles. Proteomic analysis identified several protein biomarkers related to AD in the AD animal model, including amyloid beta, tau protein, apolipoprotein E, and Triggering Receptor Expressed on Myeloid cells. Behavioral analysis demonstrated significant cognitive and memory declines in AD animal models compared with non-treated animals.

Conclusion: The intraperitoneal administration of TMT in C3H mice effectively induces pathological changes in the brain that are related to AD. The observed pathological and behavioral changes in this AD animal model resemble those found in human cases of the disease. This model can serve as a valuable platform for studying the etiology, pathogenesis, and pathophysiology of AD, as well as testing new therapies.

Keywords: Alzheimer's disease, Animal model, C3H mice, Trimethyltin.

Introduction

Alzheimer's disease (AD) is a condition that weakens the brain's ability to remember, think, and make decisions, typically affecting the elderly. The prevalence of AD is rising due to the increased human lifespan. Additionally, lifestyle changes, such as reduced physical activity, tobacco use, and other factors, can lead to diabetes, obesity, elevated cholesterol levels, and high blood pressure, which may contribute to the increasing incidence of AD not only in older adults but also in younger individuals (Durazzo *et al.*, 2014; Rapaka *et al.*, 2022).

AD is defined by the presence of senile plaques and neurofibrillary tangles (NFTs). The senile plaques are made by the aggregate of amyloid beta (Aβ) and NFTs composed of hyperphosphorylated tau protein. These

***Corresponding Author:** Hevi Wihadmadyatami. Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. Email: *heviwihadmadyatami@ugm.ac.id*

accumulations disrupt neuronal function, leading to neuroinflammation, neuronal loss, and cognitive dysfunction (Chavan *et al.*, 2023). The common symptoms of AD include memory impairment, language difficulties, visuospatial challenges, behavioral changes, and perception disturbances (Rapaka *et al.*, 2022).

Animal models are essential for studying disease pathogenesis and testing new treatments. Most currently available animal models for AD are based on the production or incorporation of β-amyloid or tau proteins, achieved through mutations or chemical injections (Smith *et al.*, 2024). Some notable AD transgenic mouse models include APP23, APP/PS1, Tg2576/TREM2 KO, 5XFAD, 3xTg, PDAPP, APPswe, TgSwDI, and APPE693A-Tg (Sethi *et al.*, 2024). In addition, streptozotocin, scopolamine, colchicine, aluminum, methionine, okadaic acid, and diazepam are chemical compounds used to induce AD in animal models (Rapaka *et al.*, 2022).

Trimethyltin (TMT) is a chemical compound that can damage nerve cells and lead to neuronal loss in specific brain regions, resulting in cognitive decline (Lee *et al.*, 2016; Onaka *et al.*, 2025). TMT has been used to induce neurodegeneration and neuronal loss using 6-month-old male Wistar rats and Sprague–Dawley rats as an animal model for AD (Nurmasitoh *et al.*, 2023; Diddi *et al.*, 2024).

In drug discovery studies, C3H mice are often used as an animal model for behavioral tests and memory recognition (Iwashita *et al.*, 2023). However, no research has utilized C3H mice induced with TMT as a model for neurodegenerative AD. This study aimed to analyze the proteomic information from blood plasma, along with routine hematology and behavioral assessments using the radial arm maze (RAM), to establish an animal model of AD from C3H mice induced with TMT.

Materials and Methods

Experimental design

The 8-week-old male C3H mice were obtained from the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada. The mice weighed 20 g. One week before treatment, the mice were placed in a controlled environment at 21°C, with a relative humidity of 45%–65%, and a 12-hour light/dark cycle. P.S. Power sample size calculation was performed, resulting in the acquisition of a total of eight mice. This study was conducted in the Laboratory Animal Experiments, Integrated Research and Testing Laboratory, Gadjah Mada University, Indonesia. The mice were randomly split into two groups: non-treated and treatment group with 2.5 mg/kg Body Weight injection of TMT (Cat# 146498, Sigma, Steinheim, Germany) in the peritoneal cavity.

Hematology analysis

Blood samples were collected from the infraorbital vein and placed in a Vacutainer EDTA tube. The blood containing Ethylenediaminetetraacetic Acid was analyzed for erythrocytes, leukocytes, hemoglobin, platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration levels. Blood markers were measured using a Sysmex KX-21 Analyzer (Sysmex, Tampines, Singapore). Statistical analysis was performed by a two-tailed unpaired t test.

Hematoxylin-eosin staining

Each mouse was anesthetized intraperitoneally with a combination of 100 mg/kg of ketamine (Cat# 17, 7421, Kepro, Maagdenburgstraat, Netherlands) and 10 mg/kg of xylazine (Cat# IX2, Interchemie, Metaalweg, Netherlands). Cervical dislocation was performed by placing the finger behind the base of the skull and firmly pulling the tail. The brain was then sequestered from the cranium by trimming the bones at the caudal end and carefully removing the skull covering the cerebellum. Brain tissue was fixed in 4% paraformaldehyde (Cat# 158127, Sigma, Steinheim, Germany).

The brain samples were preserved in paraffin blocks and sectioned into tissue slides. The slides were deparaffinized using xylene and rehydrated with absolute and graded ethanol. Each tissue slide was immersed in a hematoxylin solution (Cat# 05-M06004, Bio-Optica, Milan, Italy) for 2 minutes and then rinsed with phosphate-buffered saline. Subsequently, the slides were immersed in Eosin Y (Cat# 05-M10002, Bio-Optica, Milan, Italy) for 7 minutes. The slides were dehydrated using ethanol, cleared using xylene, and finally mounted with Canadian balsam (Cat# 101691, KgaA, Darmstadt, Germany). The samples were observed and captured using a light microscope (Olympus, BX5, Tokyo, Japan) and Optilab software (Optilab, Yogyakarta, Indonesia).

Nissl staining

Cresyl violet (Cat# C5042, Sigma, Steinheim, Germany) was used for Nissl staining according to the existing procedure. Brain slices on slides were dipped in 0.1% cresyl violet solution for 15 minutes. After immersion, the brain slides were rinsed with distilled water and then dehydrated with 70% ethanol, followed by two washes with absolute ethanol and xylene. Finally, the slides were covered with a coverslip and inspected using a light microscope at a magnification of 40× (Olympus, Tokyo, Japan). Pyramidal cells in the hippocampal region (per mm²) were counted using Optilab Software (Optilab, Yogyakarta, Indonesia).

Proteomics sample preparation

A 1.5 ml blood sample was taken from the mice and centrifuged at 2,000×*g*for 10 minutes to collect plasma. A 250 µl portion of plasma was mixed with 6 µl of 8M urea (Cat# 66612, Merck, Darmstadt, Germany) and 250 µl of 10 mM dithiothreitol (Cat# 43819, Merck, Darmstadt, Germany) in a 50 mM ammonium bicarbonate (Cat# 101131, Merck, Darmstadt,

Germany) solution and incubated for 1 hour at 30°C. The mixture was diluted with ammonium carbonate and incubated for 1 hour in the dark at room temperature before digestion with Pierce Trypsin Protease MS grade (Cat# 900305, Thermo Fisher, Massachusetts, USA) at 37°C for 16 hours. Finally, 50 µl of 1% formic acid was added, and the samples were filtered using a 0.22 µm Millipore filter.

Untargeted proteomic screening analysis using liquid chromatography-high-resolution mass spectrometry (LC-HRMS)

Analysis of proteomics was conducted using liquid chromatography (Thermo Scientific, Massachusetts, USA) and an Orbitrap High Resolution Mass Spectrometer (Orbitrap Exploris 240, Thermo Scientific, Bremen, Germany). A 5 µl sample was injected into the analytical column, specifically the Thermo Scientific Acclaim PepMap 100 C18 (Thermo Fisher, Vilnius, Lithuania). The untargeted proteomic profile was analyzed using Thermo Scientific Proteome Discoverer 2.5 and the SequestHT mass spectra database. The peptides were then compared to databases at NCBI and UniProt (https://www.uniprot.org/).

Behavioral testing using a RAM

Behavioral testing was performed in a standard RAM. The RAM was octagonal, with each side measuring 5 cm. The arms measured 5 cm in width and 30 cm in length, featuring walls that stood 10 cm tall, with a door connecting each arm to the octagon. The experiments were conducted in the same room where the mice were housed. To acclimate the mice, they were positioned facing north and in the center of the maze and allowed to search for food in their arms.

Procedure of the RAM

The testing comprised two phases: the training and trial phases (Fig. 1). The training phase was conducted over 7 days. On day 9, 1 day after TMT administration, the mice were evaluated in the trial phase. During the training phase, some maze doors were opened, allowing access only to arms 2, 4, 5, and 7 containing food. The trial test started by opening all the doors and placing the mice in the center of the maze, where they could explore until all the pellets were consumed. The trial phase lasted for 14 days, with testing scheduled for 7:00 PM. Each mouse in the training and trial phases was tested once daily for 22 days during the dark cycle. The mice were not provided with any food prior to these tests. The memory score was assisted with correct arm entries.

Ethical approval

The Research Ethics Committee of the Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia, approved the experimental protocols (Ethical Clearance No. 120/EC-FKH/Int./ 2024). The animal husbandry system adapts to its natural habitat to minimize danger, pain, and stress.

Results

Analysis of hematology in the peripheral blood profile of an AD animal model

The blood test results indicated an increase in hematocrit, MCV, and neutrophil levels, which suggests acute inflammation. However, the erythrocyte

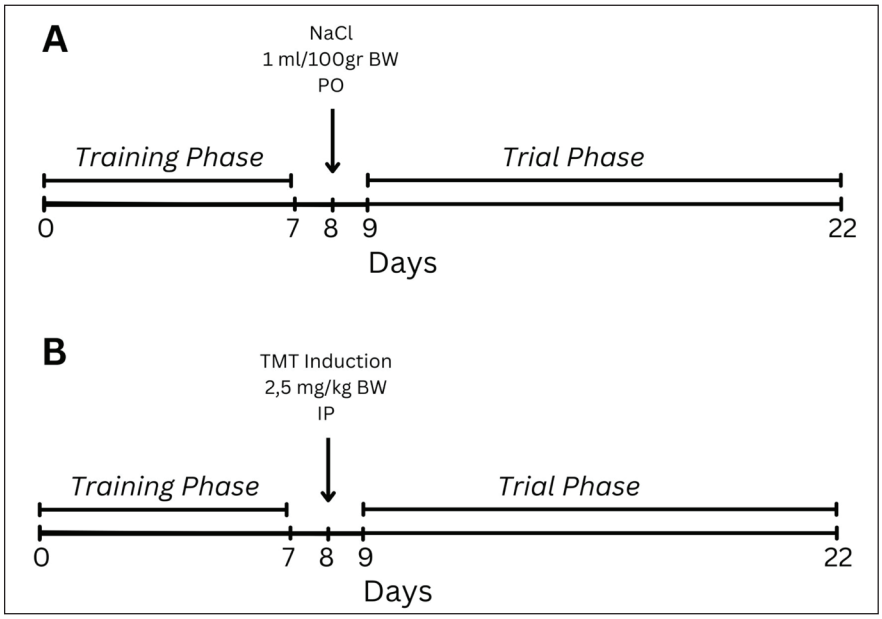

Fig. 1. The behavioral test research design utilizes a RAM and consists of two phases: the training phase and the trial test. (A) The training phase was conducted over the first 7 days before administering TMT. (B) The trial phase test was conducted from the 9th to the 22nd day, lasting for 15 days during the dark cycle (n = 4).

and hemoglobin levels were normal, indicating that there was no anemia (Table 1).

Neuropathological findings in the brain of an AD animal model with hematoxylin-eosin staining

Hematoxylin-eosin staining of the brains of normal C3H mice and C3H mice with AD showed neuroinflammation and NFTs in the cortex of the cerebrum (Table 2, Fig. 2).

Neuropathological findings in the hippocampus of an AD animal model with Nissl staining

Nissl staining focused on neuronal cell density. Our results revealed significant differences in the density of pyramidal cells in the cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) regions when comparing the tested group with TMT to the control group (Fig. 3). The administration of TMT affected the apoptosis and necrosis of neuronal cells in the hippocampus, resulting in a significant decrease in the density of these cells ($p < 0.05$) compared with the non-treated group.

Peptide sequencing of an AD animal model using LC-HRMS

Peptide sequence was conducted using LC-HRMS analysis, revealing the presence of 10 master proteins in blood plasma samples in the AD animal model that were not found in normal animals (Table 3).

Cognitive ability of AD animal model induced with TMT

We conducted a RAM experiment on TMT-induced animals to assess their cognitive ability to recall the locations of food pellets within the maze. Our study compared memory performance between a non-treated group (saline) and a treatment group. The treatment group received 2.5 mg/kg BW of TMT. Notably, the mice treated with TMT displayed a significant cognitive decline, as evidenced by repeated errors when trying to access the maze arms that held the pellets. In contrast to the non-treated group, these mice exhibited confusion for a longer duration before correctly navigating to the appropriate maze arms during the trial phase. A significant change in memory score was observed between the treatment group with TMT and the non-treated group with saline (Fig. 4).

Discussion

AD is marked by memory loss and a decline in cognitive function due to pathological changes in the brain. C3H mice have aggressive, hyperactive, and increased anxiety and tend to avoid open spaces. C3H mice also have cognitive abilities in learning and memory, making them ideal as experimental animals for studying cognitive disorders and the impact of drugs on cognition (Bates *et al.*, 2014). In this study, we aimed to investigate the use of TMT chloride to develop an animal model of AD. Mechanism of AD induction by TMT involves by an inflammatory reaction by activating astrocytes and microglia to release inflammatory cytokines, oxidative stress, and mitochondrial dysfunction, leading to neuronal loss in the central nervous system (Lee *et al.*, 2016; Chavan

Table 1. Description of haematology in AD animal model C3H mice ($n = 4$) (*$p < 0.05$; n.s = not significant).

Parameter	Units	Reference	Non-treated group	Treatment group	Result (TMT *vs.* Reference)	Result (NT *vs.* TMT)	Two-tailed unpaired test (NT *vs.* TMT)
Hematocrit (PCV)	%	35.0–40.0[a]	39.1 ± 2.68	41.6 ± 0.60	Increase	**Increase**	ns
Erythrocytes	10^6 sel/µl	4.22–9.93[b]	7.16 ± 1.48	7.24 ± 0.08	Normal	Normal	ns
Hemoglobin	g/dl	6.70–16.00[b]	11.6 ± 0.87	12.1 ± 0.37	Normal	Normal	ns
MCV	fl	47–52[c]	53.1 ± 0.75	54.6 ± 0.17	**Increase**	**Increase**	ns
MCH	ρg	14.7–18.4[c]	16.2 ± 0.20	16.7 ± 0.30	Normal	Normal	ns
MCHC	g/dl	29.5–37.7[c]	29.7 ± 0.35	30.6 ± 0.60	Normal	Normal	ns
PLT	10^3 sel/µl	834–1290[a]	1125 ± 75.44	1119 ± 80.65	Normal	Normal	ns
Leukocyte	10^3 sel/µl	5.50–11.60[a]	6.8 ± 1.65	7.8 ± 1.57	Normal	**Increase**	*
Neutrophils	10^3 sel/µl	3.06 ± 0.49[d]	2.9 ± 0.78	4.1 ± 1.38	**Increase**	**Increase**	ns
Lymphocytes	10^3 sel/µl	5.46 ± 0.3[d]	3.9 ± 0.83	3.5 ± 0.30	**Decrease**	Normal	ns
Monocytes	10^3 sel/µl	0.26 ± 0.03[d]	0	0	Normal	Normal	ns

[a]Barrios , M., Rodruguez-Acosta, A., Gil, A., Salazar, A.M., Taylor, P., Sanchez, E.E., Arocha-Pinango, C.L. and Guerrero, B. 2009. Comparative hemostatic parameters in BALB/c, C57BL/6 and C3H/He mice. Thromb. Res. 124, 338–343.

[b]Mazzaccara, C., Labruna, G., Cito, G., Scarfo, M., Felice, M.D., Pastore, L. and Succhetti, L. 2008. Age-related reference intervals of the main biochemical and hematological parameters in C57BL/6J, 129SV/EV and C3H/HeJ mouse strains. PLoS One 3(11), e3772; doi:10.1371/journal.pone.0003772

[c]Jacobsen, K.O., Villa, V., Miner, V.L. and Whitnall, M.H. 2004. Effects of anesthesia and vehicle injection on circulating blood elements in C3H/HeN male mice. Contemp Top Lab Anim Sci. 43(5).

[d]Aigner, B., Rathkolb, B., Klempt, M., Wagner, S., Michel, D., Klaften, M, Laufs, J., Schneider, B., Sedlmeier, R., de Angelis, M.H. and Wolf, E. 2011. Generation of N-ethyl-N-nitrosourea-induced mouse mutants with deviations in hematological parameters. Mamm. Genome. 22, 495–505.

Bold values indicate differences between treatment and control groups. Significant difference is indicated by * ($p < 0.05$); ns = not significant.

et al., 2023). However, the administration of TMT to the rat could result in gliosis without disruption of the blood-brain barrier (BBB), making it ideal for the induction of pathological changes in the brain that lead to AD (Little *et al.*, 2002). In this study, we describe new evidence on TMT-induced C3H mice. We found that the intraperitoneal administration of TMT can simultaneously increase inflammation with neutrophilia, pathological changes in the brain and proteomics in blood plasma, and behavioral changes, especially in learning and memory.

Table 2. Pathological changes were observed in the brain of an AD animal model ($n = 4$).

Types of changes	Non-treated group	Treatment group
Neurofibrillary tangle	–	+
Inflammation	–	+
Senile plaque or amyloid beta plaque	–	–
Granulovacuolar bodies	–	–
Hirano bodies	–	–
Reduced in pyramidal cells	–	+

– = undetected, + = detected.

Hematology analysis revealed a greater value in hematocrit, MCV, leucocyte, and neutrophil levels in the treatment group (TMT 2,5 mg/kg) compared to the non-treated group. The leucocyte level was significantly different between the non-treated and treatment groups ($p < 0.05$). The elevation of leucocytes, especially in neutrophils, may be attributed to neuroinflammation caused by high oxidative stress in the brain, linked to Aβ plaques (Huang *et al.*, 2022). Tumor necrosis factor-alpha is first induced following the activation of

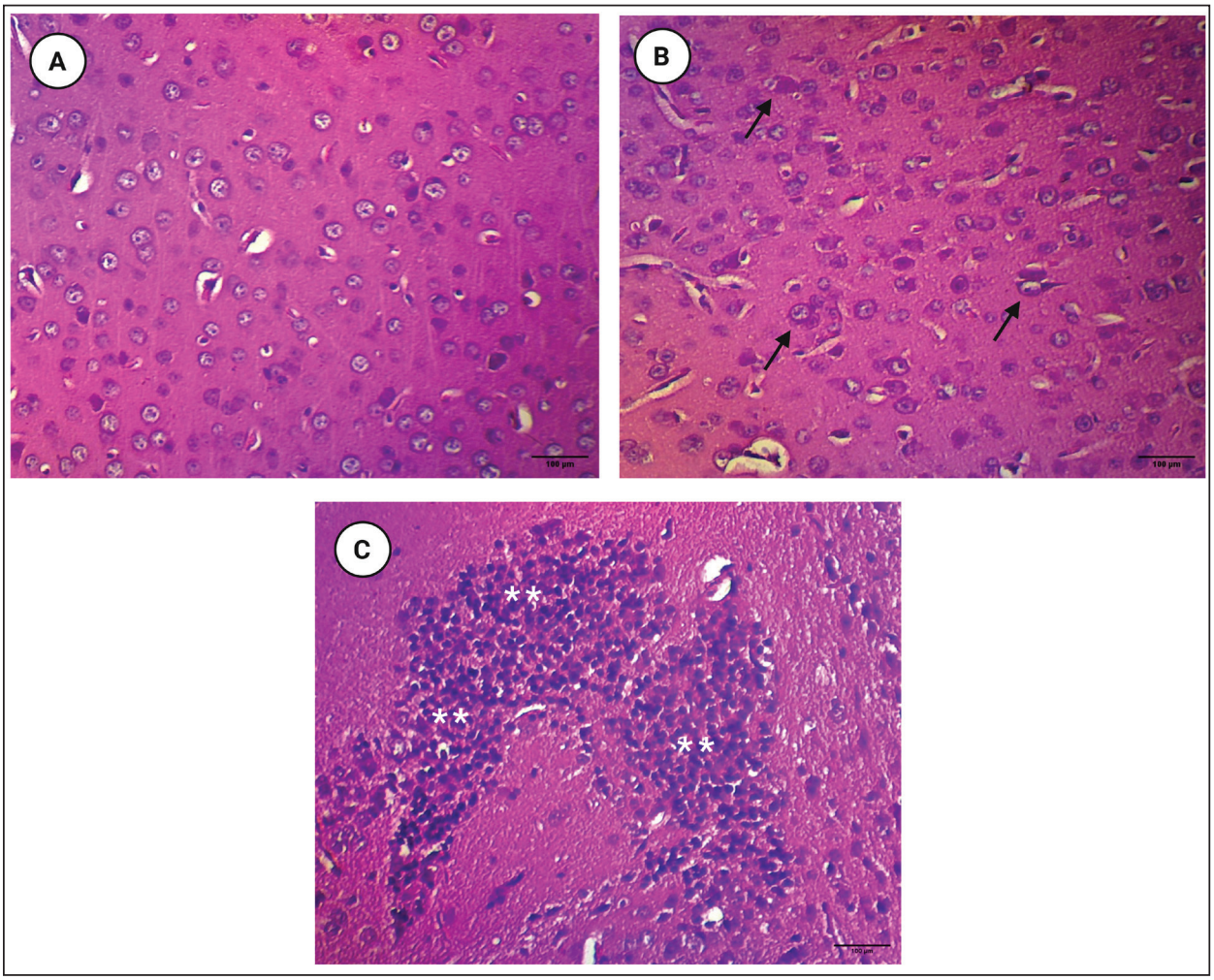

Fig. 2. Histology of the brain of non-treated group (normal) mice versus treatment group with TMT ($n = 4$) (H&E). (A) Normal mice receiving saline showed a normal histological structure of the cerebral cortex (B), NFTs (arrow) in the TMT treatment group, and (C) neutrophilic and lymphocytic infiltration (asterisk) in the TMT treatment group ($n = 4$).

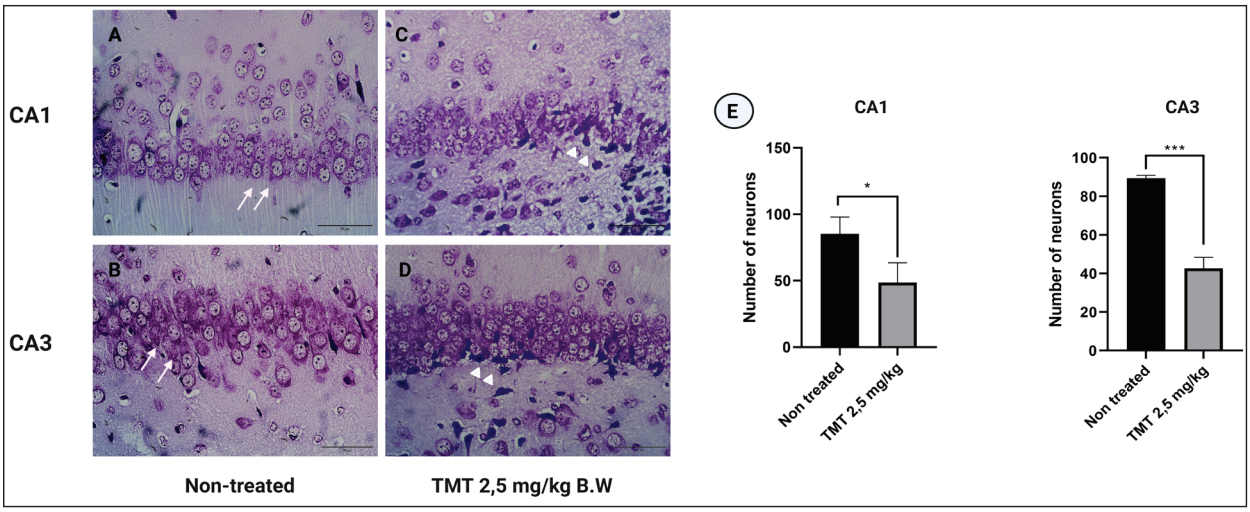

Fig. 3. TMT injection leads to morphological changes in the cells of the hippocampus of mice. The morphology of pyramidal cells in the hippocampus was assessed using Nissl staining. (A & B) The histology of the hippocampus in the normal/non-treated group. The normal formation of the hippocampus exhibits a clear layer of pyramidal cells with uniform morphology of the CA1 and CA3 regions (white arrow). (C & D) The histology of the hippocampus in the TMT treatment group. Nissl staining revealed a disordered pyramidal cell layer. The pyramidal cell appeared shrunken and exhibited a dark stain (white arrowheads). (E) Quantification of pyramidal cells in CA1 and CA3 of the hippocampal regions in a mouse model of AD. Statistical analysis was performed by a two-sided unpaired *t*-test ($n = 4$; */**/***; significant ($p < 0.05$); n.s, not significant).

Table 3. Result of blood plasma proteomic analysis of animal model of AD with C3H mice ($n = 4$).

No	Name	Coverage	Peptides	MW [kDA]	Non-treated group	Treatment group
1	Amyloid beta (A4)-like protein 2	3	1	79	-	✓
2	J Chain J, Isoform Tau-E of Microtubule-associated protein tau	14	1	5.9	-	✓
3	Apolipoprotein A-IV	5	1	49.2	-	✓
4	Apolipoprotein B	1	1	503.6	-	✓
5	Apolipoprotein C-III	14	1	15.2	-	✓
6	apolipoprotein E	4	1	36.2	-	✓
7	apolipoprotein E receptor 2	4	1	109.8	-	✓
8	Triggering receptor expressed on myeloid cells 3 (TREM3)	1	1	20.4	-	✓
9	tumor necrosis factor alpha-induced protein 3 isoform 3	4	1	95.5	-	✓
10	Tumor necrosis factor, alpha-induced protein 2	4	1	73.6	-	✓

microglia and astrocytes. This is followed by the release of interleukin-1 (IL-1) and IL-1β, which upregulate neutrophil activity as part of the immune response (Lee *et al.*, 2016; Huang *et al.*, 2022).

Blood is accessible and non-invasive for investigating the biomarkers of AD, but it is also challenging due to the complexity of proteomics. The α-synuclein, β-aggregates, and plaque accumulation in the central nervous system can enter blood vessels. Amyloid plaques, NFTs, Trem2, and interleukins in blood can be used as biomarkers for AD (Tin *et al.*, 2023). In the AD animal model in C3H mice, the proteomics we found were Aβ, protein tau, a family of apolipoproteins, and TREM3. These findings are consistent with those of previous studies (Banks *et al.*, 2016; Tin *et al.*, 2023; Tokuoka *et al.*, 2024). However, our study has two novelties. TMT injection was conducted in 8-week-old C3H mice (versus in C57BL/6 mice (Jeong *et al.*, 2021) and using blood plasma for proteomic analysis (versus using hippocampal (Zakaria *et al.*, 2025). Studies show

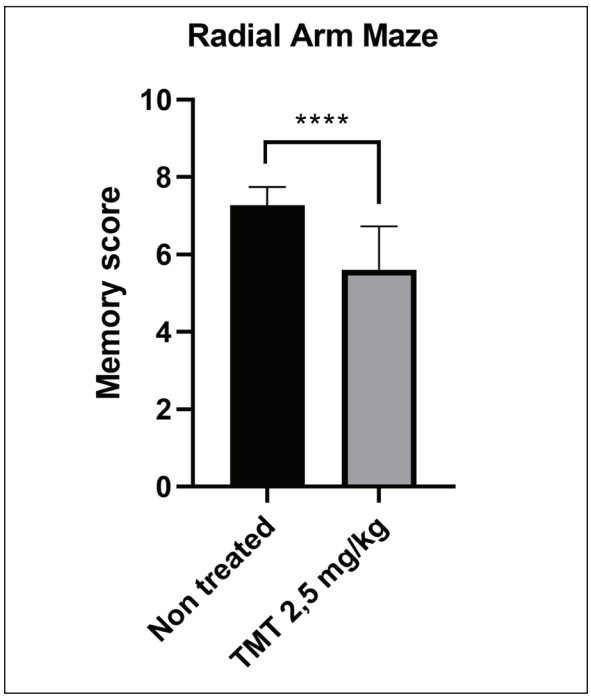

Fig. 4. Memory score. The memory score was calculated using the correct arm entries in the trial phase. The data were grouped into two groups: the non-treated group and the treatment group (TMT 2.5 mg/kg body weight). Statistical analysis was conducted using a two-tailed unpaired *t*-test. TMT induction in C3H mice can significantly reduce learning and memory abilities compared with the non-treated group ($n = 4$; */**/***; significant ($p < 0.05$); n.s, not significant).

that circulating plasma Aβ peptides in geriatric patients correlate with incident dementia (Tin *et al.*, 2023). Neurofibrillary tangles are caused by a tau-associated microtubule protein. Tau proteins can cross the BBB in both directions (Banks *et al.*, 2016). Using LC-HRMS, we identified several apolipoproteins, including apolipoproteins A, B, C, and E. Apolipoproteins are crucial for lipid transport and metabolism. The presence of genetic variations in apolipoprotein DNA sequences is one of the common genetic factors associated with late-onset AD. Apolipoproteins are also associated with other disease variants, including those related to cardiovascular issues and neurodegeneration (Tokuoka *et al.*, 2024). Triggering Receptor Expressed on Myeloid cells are cell-surface receptors found mainly on granulocytes, monocytes, and macrophages. They play a role in the development of neurodegenerative diseases, inflammation, metabolic syndrome, atherosclerosis, and cancer (Colonna, 2023).

The pathological examination using hematoxylin and eosin staining, along with cresyl violet staining, revealed several changes in the hippocampus of the brains of mice. The hippocampus is a part of the brain that functions in learning and memory (Rao *et al.*, 2022). Our study found that the pyramidal cell layers became disordered in the animal model of AD. Some of the pyramidal cells appear shrunken and exhibit dark stains related to apoptotic and necrosis cell death. TMT typically induces neuronal death in pyramidal cells in the cornu ammonis, occurring two days after intraperitoneal injection (Geloso *et al.*, 2011; Onaka *et al.*, 2018). Our research revealed the occurrence of NFT accumulation and inflammation in the brain of the AD mouse model. Neurofibrillary tangles are structured bundles of tau protein that form near the affected neurons' cell surface. Glial cells, particularly microglial cells, react to the presence of NFTs and produce an inflammatory response (Wilcock, 2012; DeTure and Dickson, 2019).

It also shows a linear relationship with cognitive ability following TMT injection. The RAM was performed to assess memory capabilities in mice. After TMT injection, we found that the mice had difficulty remembering the correct arm that contained the pellets and had been previously tested in the training phase. The mice AD model also made errors in entering the arm that did not contain pellets. Previous studies have shown that intraperitoneal administration of TMT in mice can cause neurological lesions and impair acetylcholine function within the cholinergic system, which is linked to memory, cognitive decline, and learning ability and leads to behavioral disorders (Tu *et al.*, 2017; Chen *et al.*, 2022; Ye *et al.*, 2024).

Conclusion

TMT administration to C3H mice successfully induced neurodegeneration resembling AD. This included nerve cell death, neuroinflammation, and NFTs in the brain, changes in blood parameters, and the presence of Alzheimer's biomarkers in blood plasma. Behavioral changes, such as memory loss and decreased learning ability, were also observed. Histopathological analyses revealed structural changes in the cortex of the cerebrum and hippocampus, and leukocytosis, particularly neutrophilia, was observed in blood tests. Proteomic testing identified specific biomarkers associated with AD, including Aβ, tau protein, apolipoprotein E, and TREM3. Behavioral assessments indicated declines in learning and increased aggression.

This study has a number of limitations. First, the histopathological changes observed do not encompass all the pathological alterations in the brain associated with AD in humans. Second, the study duration was only 15 days, which does not adequately represent the chronic development of TMT as an inducer of AD. Third, the small sample size limits the generalizability of the results. Additionally, the proteomic findings lack definitive information regarding the relationship between TMT and the function of the biomarkers.

Future research should focus on study duration reaching chronic stages, a larger sample size, and testing biomarkers that correlate with AD.

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Conflict of interest

All authors declare no conflict of interest.

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Authors' Contribution

Conceptualization, HW, DLK DA; Methodology, HW, DLK, DAAN, DA; Validation, HW, DLK, WTW; Formal analysis, WBTS, APA, UK, MZZR, DA; Investigation, WBTS, APA, DA, MZZR; Resources, HW, DLK, DA DAAN; Data curation, DA, MZZR, APA, WBTS; Writing-original draft preparation, HW, MZZR, DA, SK; Writing-review editing, HW, APA, DA; Visualization, DA, APA, MZZR, WTW; Supervision, HW, DLK, DAAN; Project administration, HW; Funding acquisition, HW.

Data availability

The manuscript includes all the data supporting the findings of this study.

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