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Animal model for sporadic dementia of Alzheimer's type (SDAT) using streptozotocin and lipopolysaccharide combinations in rats

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ABSTRACT

Sporadic dementia of Alzheimer's type (SDAT) pathogenesis has not been revealed completely due to the difficulty in creating an appropriate animal model. The purpose of this study was to investigate the effect of single-dose intraperitoneal (IP) induction of streptozotocin (STZ) and lipopolysaccharide (LPS) on the β-amyloid levels and the brain function of experimental rats. Eighteen rats were divided into three groups i.e. control, TRE1 (STZ 60 mg.kg^{-1} BW + LPS 3 mg.kg^{-1} BW), and TRE2 (STZ 30 mg.kg^{-1} BW + LPS 1.5 mg.kg^{-1} BW). The substances were administered in a single dose. Behavioral tests were started at day-30 after injection, we performed Morris water maze (MWM) and novel object recognition (NOR) tests. Afterward, we measured whole brain and serum β-amyloid levels, as one of the biomarkers of Alzheimer's Disease (AD), using the ELISA method. In MWM tests, the escape latency and time spent in the target quadrant of treatment groups were significantly higher than those in control at the day-5 MWM test and probe trial. The rats in treatment groups have negative discrimination indexes in NOR tasks, indicating that the rats could not remember the familiar object. Intraperitoneal STZ and LPS significantly increase soluble brain β-amyloid levels of treatment groups than those in the control group. In conclusion, the treatment of STZ (60 mg.kg^{-1} BW) and LPS (3 mg.kg^{-1} BW) indicated spatial and recognition memory impairment, along with an increase of brain soluble β-amyloid level in rats.

ABSTRAK

Patogenesis tipe demensia Alzheimer sporadis (SDAT) belum terungkap sepenuhnya karena hambatan dalam menemukan hewan model yang sesuai. Tujuan penelitian ini adalah untuk mengkaji efek induksi streptozotocin (STZ) dan lipopolisakarida (LPS) secara intraperitonial (IP) pada tingkat β-amiloid dan fungsi otak tikus percobaan. Delapan belas tikus dibagi menjadi tiga kelompok yaitu kontrol, TRE1 (STZ 60 mg.kg^{-1} BB. + LPS 3 mg.kg^{-1} BB), and TRE2 (STZ 30 mg.kg^{-1} BB + LPS 1.5 mg.kg^{-1} BB). Zat tersebut diberikan dalam dosis tunggal. Uji perilaku dimulai pada hari ke-30 setelah injeksi, menggunakan uji *Morris water maze* (MWM) dan *Novel object recognition* (NOR). Setelah itu, kadar β-amiloid pada otak dan serum diukur menggunakan metode ELISA sebagai salah satu biomarker penyakit Alzheimer (AD). Hasil penelitian menunjukkan bahwa latensi dan durasi tikus di kuadran target untuk kelompok perlakuan secara signifikan lebih tinggi daripada tikus kontrol pada uji MWM hari ke-5 dan uji *Probe trial*. Tikus dalam kelompok perlakuan memiliki indeks diskriminasi negatif dalam uji NOR, menunjukkan bahwa tikus tidak dapat mengingat objek yang dikenalnya. STZ dan LPS secara signifikan meningkatkan kadar β-amiloid otak pada kelompok perlakuan dibandingkan kelompok kontrol. Dapat disimpulkan, perlakuan STZ (60 mg.kg^{-1} BB) dan LPS (3 mg.kg^{-1} BB) menyebabkan gangguan memori spasial dan rekognisi, serta meningkatkan kadar β-amiloid terlarut pada otak tikus.

Keywords:
β-amyloid;
lipopolysaccharide;
memory;
sporadic dementia;
streptozotocin;

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INTRODUCTION

Dementia is generally described as a cognitive impairment that is severe enough to disturb social or job-related brain function.[1] According to the cause of dementia, there are four common subtypes of dementia-Alzheimer's disease (AD), vascular, frontotemporal, and Lewy body dementia.[2] The most common cause of dementia is AD, which is estimated at 60 to 80% of cases.[3] The first symptom of dementia is memory dysfunction, a weakening in ability for consolidation and recall of newly received information.[4] Disorder of episodic and semantic memory systems are the main indication of AD.[5,6] Hippocampus, that crucial in episodic memory system, is reduced its volume in AD.[7] Memory deterioration in AD tends to be anterograde amnesia subsequently retrograde amnesia, the lack of ability to create novel memories and regain old memories, respectively.[8]

More than 90% of patients with AD seem to be sporadic and to have old onset.[9] Amyloid protein precursor (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2) mutations are involved in early-onset AD pathogeny.[10] Typical late-onset AD is probably due to the complex interaction between genetic and environmental factors.[11] Several hypotheses have been proposed to explain AD pathogenesis - cholinergic, amyloid, tau protein, and apolipoprotein ε4 hypothesis.[12,13] Among others, β-amyloid plaque and neurofibrillary tangles are implicated in AD pathogenesis and is a key element of the present etiology hypotheses.[11]

Commonly, the laboratory animal models are established on the familial AD-linked mutations that yielding in Aβ level increase and accumulation.[14] Meanwhile, transgenic models for sporadic AD, such as the APOE ε4 gene mutation in mice and those in primates, are severely limited by availability issues and costs. Since 90% of cases of AD are sporadic, the opportunity for developing non-GMO animal models is wide open.

Streptozotocin is first known for its anti-cancer activity, then being used as an induction agent to create a model for diabetes.[15] Streptozotocin moves into pancreatic beta-cell, induce DNA alkylation, and eventually generate cell necrosis.[16] Cellular toxicity of STZ is supposed to be facilitated by increasing reactive oxygen species (ROS) in mitochondria that stimulate the oxidative stress of the cells.[17] Several metabolic pathways known to induce these cellular stress are polyol pathway, hexosamine pathway, diacylglycerol-activated protein kinase C (PKC), and advanced glycation end products (AGE) formation.[18]

Streptozotocin enters the cell via protein transporter GLUT2, the main glucose carrier in several organs, including the brain.[19,20] The particular mechanism on how STZ affecting the brain is still being investigated. Moreover, the effect of combination of intraperitoneal STZ and LPS on brain function is still unknown. The study aimed to investigate the effect of single-dose intraperitoneal (IP) induction of STZ 60 $mg.kg^{-1}$ BW and lipopolysaccharide (LPS) 3 $mg.kg^{-1}$ BW on the β-amyloid levels and the brain function of experimental rats as a model of sporadic dementia.

MATERIALs AND METHODS

Animals

Wistar rats (*Rattus norvegicus* Berkenhout, 1769) from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta were housed at colony cages contained wood chip bedding with standard pellet food and tap water ad libitum. The room environment was maintained in constant temperature (26-27°C) and humidity (76-88%) on a 12 h light/dark cycle. The

protocol of study was approved by the Research Ethic Committee for Preclinical Research, Universitas Gadjah Mada (Ref. 00030/04/LPPT/VI/2019).

Materials

Streptozotocin was obtained from Cayman Chemical (Ann Arbor, MI, USA) and was dissolved in a citrate buffer. Lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ketamine was obtained from Troy Laboratories (Glendenning, NSW, Australia) and Xylazine was obtained from Interchemie (Metaalweg, Venray, Holland). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Animal treatments

Rats were separated into three groups, each containing six rats. The control group (CNT) was given 1 mL distilled water (placebo), the second group of rats received IP injection of STZ (60 $mg.kg^{-1}$ BW) and LPS (3 $mg.kg^{-1}$ BW), meanwhile the third group of rats received IP injection of STZ (30 $mg.kg^{-1}$ BW) and LPS (1.5 $mg.kg^{-1}$ BW). All substances mentioned were administered using a 27 G syringe in a single dose.

MWM test

Morris water maze (MWM) tasks were performed in a black-colored circular tank (diameter, 1.5 m) filled with tap water (30-32 ^{o}C). The start or finish platform was built from 10 x 10 x 15 cm clear glass cube. The swimming path was recorded using video camera B-PRO5α fixed focus F2.8 f= 3 mm 170^{o} wide-angle lens (Brica, China) and analyzed using Idtracker 2.1 (Cajal Institute, Madrid, Spain). The task was carried out for five consecutive days consisted of training trial, probe trial, and visual cue trial.[21] For day one training, the rat was located in the tank at four random quadrants with a visible platform. On day two until four, training trials were performed with the hidden (submerged) platform. The latency for individual training was documented. The probe test was conducted on day 5. In the probe trial, rat was left to dip in the tank for one min devoid of the platform. The duration in the target (platform location) quadrant was noted. In the visual cue test, the platform was returned into the visible condition and the latency was recorded.

NOR test

Novel object recognition (NOR) tasks were carried out for four days which included familiarization, habituation, and test day.[22] In familiarization, a rat was placed in the test cage box (40 x 40 x 40 cm, black colored) for 5 min. In the subsequent day, the habituation was performed by introduced a rat with two identical objects in 5 min duration for two consecutive days. Then the test day was done by replacing one object with another object of different shape and color. The duration of the test was limited to 60 sec. Discrimination index (DI) was quantified by comparing the duration spent by the rat to sniff on new objects and that on the initial object.

Blood collection

Blood and brain samples were collected after behavioral tests. Before blood and brain collection, animals were anesthetized with i.m. ketamine (50 $mg.kg^{-1}$ BW) and xylazine (20 $mg.kg^{-1}$ BW) cocktail. Whole blood samples were collected from retro-orbital sinus, coagulated for 15 min, and spun using Centrifuge 5418R (Eppendorf, Hamburg, Germany) at 4,000 rpm for 15 min at 4^{o}C. The supernatant was collected and stored under -20 ^{o}C for further analysis.

Blood glucose level and body weight

Blood glucose levels and body weights of rats were measured on the day-30 after STZ-LPS administration. Plasma glucose levels using amperometric glucose meter Easytouch GCU (Bioptik Technology, Taiwan). Meanwhile, body weights were measured using an analytical balance (Ohaus Instrument, Shanghai, China).

Brain collection

For brain collection, anesthetized animals were dissected until the heart exposed. Perfusion was then performed with a cannula located in the cor (left ventricle). Afterward, the right atrium was incised and the descending thoracic aorta was clamped. Perfusion was done using cold phosphate-buffered saline with heparin sodium (PT. Pratapa Nirmala, Tangerang, Indonesia) at 1,000 $IU.mL^{-1}$ concentration. Subsequently, animal skulls were opened until the brain completely exposed. The whole brain was isolated and kept on ice for further homogenization.

β-amyloid extraction

β-amyloid extraction was performed based on the modified β-amyloid extraction protocol by Casali and Landreth.[23] Fresh isolated whole brain tissues were mechanically homogenized using Handheld Homogenizer MT-13K (Huangzhou Miu Instrument Ltd, Zhejiang, China) at 18,000 rpm for 2 min in cold 850µL tissue homogenization buffer (2 mM Tris and pH 7.4); 250 mM sucrose; 0.5 mM EDTA (Ethylenediaminetetraacetic acid); 0.5 mM; H_2O) containing fresh protease inhibitor cocktail on ice. For soluble β-amyloid extraction, 250µL brain homogenates were added with 250 µL 0.4% diethylamine then spun at 14,000 rpm for 1 h at 4°C. Obtained supernatants (extracts) were neutralized with 42.5 µL 0.5 M Tris-HCl (pH 6.8), were frozen in liquid nitrogen, and were kept in the freezer for soluble β-amyloid analysis. Meanwhile, the pellet homogenates were added with 125µL cold formic acid, were mixed using a vortex, and were spun at 14,000 rpm for 1 h at 4°C. Afterward, 105µL supernatants (extracts) were mixed with 1.895mL of formic-acid neutralization buffer (1 M Tris base; 0.5 M Na_2HPO_4; 0.05% NaN_3), were frozen in liquid nitrogen, and were kept at the freezer for insoluble β-amyloid analysis.

β-amyloid quantification

Frozen serum and brain extracts were thawed before performing the assay. Serum and brain-derived β-amyloid were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) method, according to assay procedure of Fine Test ELISA Rat Amyloid-β 40 Kit (Wuhan Fine Biological Technology, Hubei, China) with catalog number: ER0754. Samples were analyzed in duplo. Optical density (OD value) of standards and samples were measured using a Microplate Reader Elx800 (Biotek Instruments, Vermont, USA) set to 450 nm.

Statistical analysis

All data were statistically evaluated using SPSS 22.0 (IBM corporation, USA). We used the oneway ANOVA (analysis of variance) and Tuckey test to analyze MWM latencies, NOR index, soluble brain β-amyloid, blood glucose levels, and body weights. Meanwhile, we used Kruskal-Wallis test and Kruskal-Wallis Pairwise Comparison for soluble brain β-amyloid and serum β-amyloid. The results were considered significant at $p<0.05$.

RESULTS

MWM test

In the MWM test, rats form a spatial memory using visual stimuli around the chamber. Memory was assessed by the duration before the animal find the platform (escape latency) and by the proportion of the duration in the platform area (target quadrant). Escape latencies of the rats in each group were observed during the MWM test. Latency data were normally distributed. The results of the ANOVA and Tuckey test showed there was a significant variation in escape latency at training day-5 (FIGURE 1A). The escape latencies in the TRE1 rats were significantly longer than those in the CNT group ($p<0.05$). The swimming trajectories of the rats in the CNT group were smooth and direct into the escape platform. Meanwhile, the swimming trajectories of TRE1 and TRE2 rats were disorganized (FIGURE 2A).

The probe trial was conducted on training day-5 to assess spatial memory. Data were normally distributed. The time spent in the target quadrant of TRE1 and TRE2 rats were significantly shorter than those in CNT rats ($p<0.05$) according to the ANOVA and Tuckey test (FIGURE 1B). Movement trajectories of rats at the MWM Probe Trial showed a disorganized path in TRE1 and TRE2 rats (FIGURE 2B).

To asses that MWM results were not affected by sensorimotor function, we used the MWM visual cue test. The visual cue test was conducted after the MWM probe trial on day 5 of training. Latency data were normally distributed. The ANOVA analysis showed no significant sensorimotor function effects on visual cue parameters (FIGURE 1A).

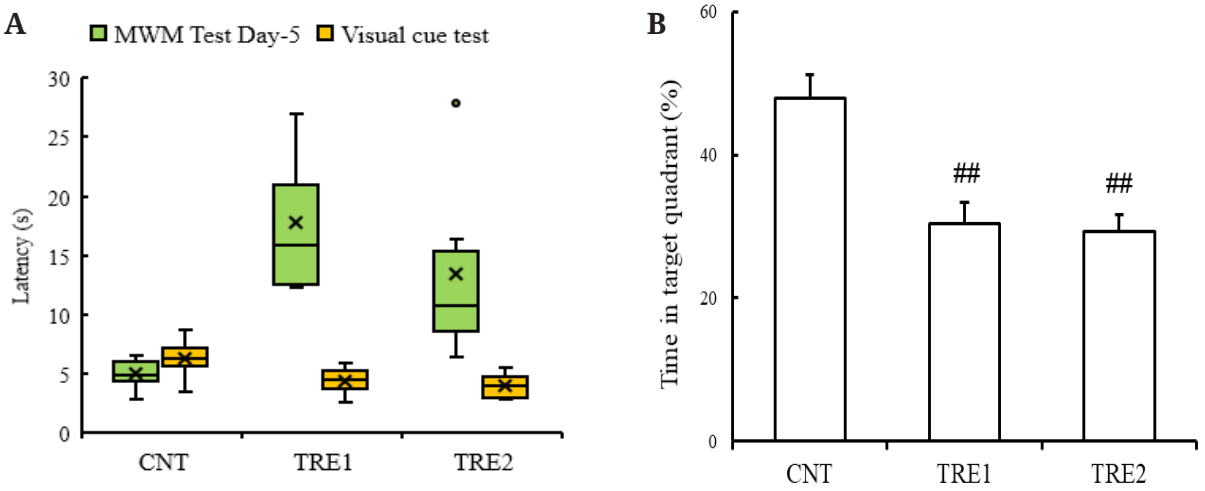

FIGURE 1. Escape latencies and time in target quadrant in MWM tasks. (**A**) Escape latencies of TRE1 rats were significantly longer than those in CNT rats at the day-5 MWM test using an invisible platform (##, $p<0.05$, ANOVA, Tuckey Test). Meanwhile, escape latencies during Visual Cue Test using MWM visible platform were not significant among all groups of treatments ($p>0.05$, ANOVA). (**B**) Time spent in the target quadrant of TRE1 and TRE2 rats were significantly shorter than those in CNT rats at MWM Probe trial (##, $p<0.05$, ANOVA, Tuckey test). CNT: control group; TRE1: STZ 60 mg.kg^{-1} BW and LPS 3 mg.kg^{-1} BW; TRE2: STZ 30 mg.kg^{-1} BW and LPS 1.5 mg.kg^{-1} BW. Data are presented as mean (×) and median with upper and lower quartiles, min and max values and outliers (o). n=6.

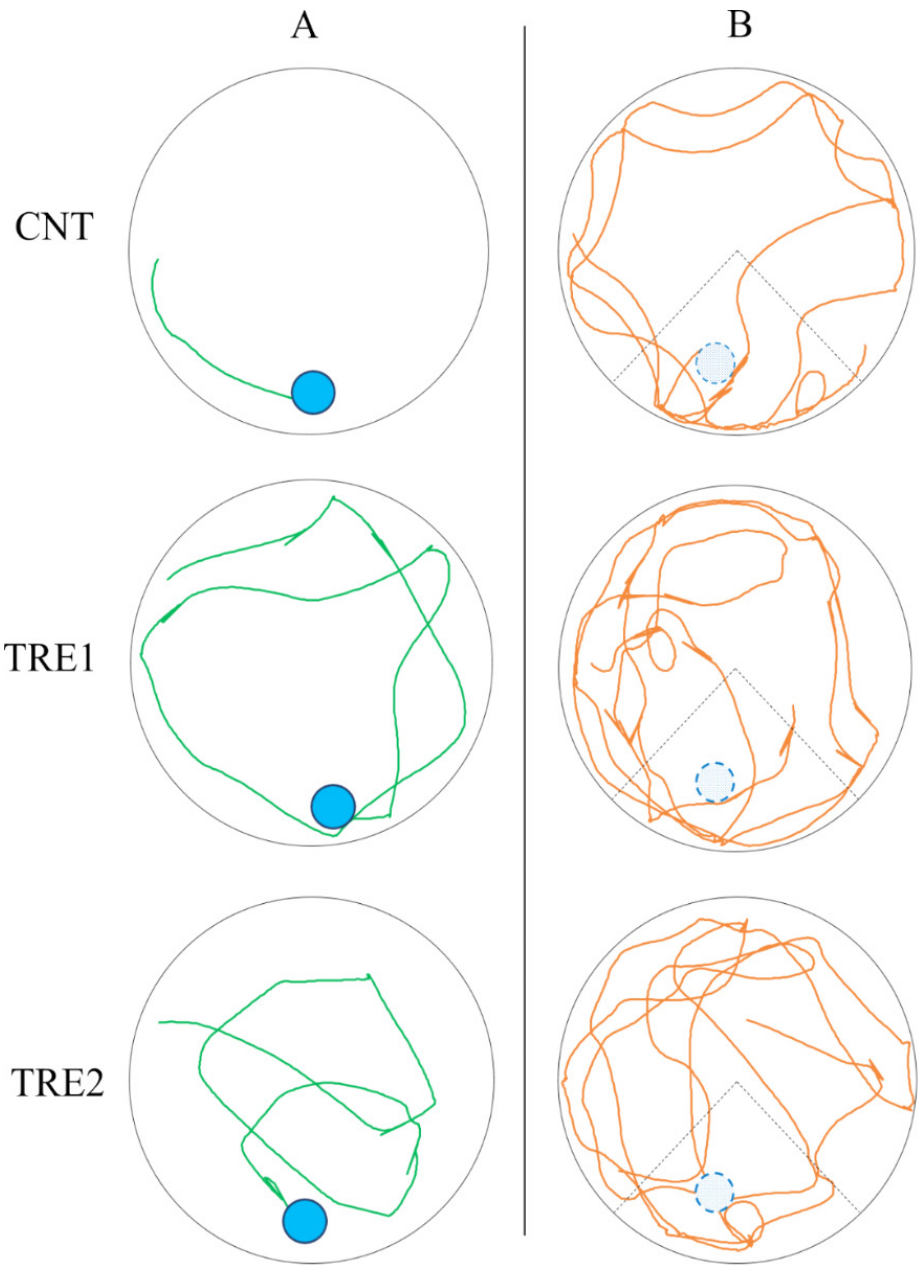

FIGURE 2. Representative movement trajectories of rats in all groups during MWM tasks. (**A**) The swimming trajectories of the rats in the CNT group were smoother and more direct than those in other groups that were disorganized at MWM Day-5. (**B**) Movement trajectories of rats at MWM Probe Trial showed a disorganized path in TRE1 and TRE2 rats. Platform was removed during Probe Trial. CNT: control group; TRE1: STZ 60 $mg.kg^{-1}$ BW and LPS 3 $mg.kg^{-1}$ BW; TRE2: STZ 30 $mg.kg^{-1}$ BW and LPS 1.5 $mg.kg^{-1}$ BW.

NOR test

In the NOR test, the variations in the exploration duration of new (unfamiliar) and familiar objects were observed. We measured it using DI i.e. the deviation in the exploration period of a novel (unfamiliar) and familiar object, then comparing the score by the entire duration of exploration. DI of TRE1 and TRE2 rats showed negative scores, lower than those in CNT rats ($p < 0.05$) (FIGURE 3).

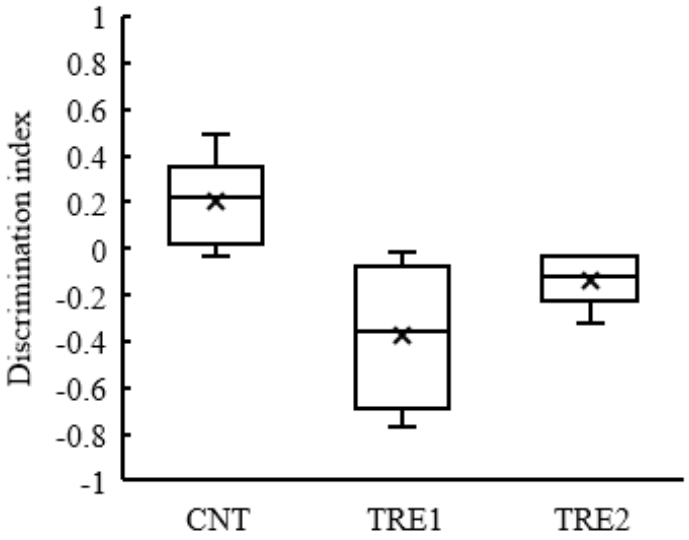

FIGURE 3. Discrimination index of TRE1 and TRE2 rats showed negative score, lower than those in CNT rats during NOR test (##, p<0.05, ANOVA, Tuckey test). CNT: control group; TRE1: STZ 60 mg.kg-1 BW and LPS 3 mg.kg-1 BW; TRE2: STZ 30 mg.kg-1 BW and LPS 1.5 mg.kg-1 BW. Data are presented as mean (×) and median with upper and lower quartiles, min and max values and outliers (o). n=5 for all groups.

Blood glucose level and body weight

Blood glucose contents and body weights of each group were measured on the day-30 after STZ-LPS administration (TABLE 1). Circulating glucose levels of the TRE1 rats were significantly higher than those in CNT and TRE2 rats (p<0.05). Meanwhile, the body weights of the TRE1 rats were significantly lower than those in other groups (p<0.05).

TABLE 1. Blood glucose and body weight of rats in all groups (mean ± SEM.)

Groups	Blood glucose ($g.dL^{-1}$)	Body weight (g)
CNT	113.33 ± 19.40	191.48 ± 5.36
TRE1	198.00 ± 30.83 ##	149.44 ± 13.01 ##
TRE2	71.67 ± 9.00 n.s	187.53 ± 6.57 n.s

CNT: control group; TRE1: STZ 60 $mg.kg^{-1}$ BW and LPS 3 $mg.kg^{-1}$ BW; TRE2: STZ 30 $mg.kg^{-1}$ BW and LPS 1.5 $mg.kg^{-1}$ BW; ##: significant to CNT (p<0.05 ANOVA, Tuckey test); n.s.: not significant to CNT; n=6

β-amyloid level

Soluble and insoluble brain β-amyloid levels in each group of rats were measured (TABLE 2). Soluble brain β-amyloid levels in TRE1 rats (509 ± 41 pg.mL^{-1}) and TRE2 rats (536 ± 36 pg.mL^{-1}) were significantly higher than those in CNT rats (326 ± 44 pg.mL^{-1}), according to ANOVA and Tuckey test ($p<0.05$). Data were normally distributed. The highest Individual soluble brain β-amyloid level was 750.09 pg.mL^{-1} (TRE1). Meanwhile, insoluble β-amyloid levels in TRE1 rats (324 ± 33 pg.mL^{-1}) were higher than CNT rats (287 ± 18 pg.mL^{-1}) and TRE2 rats (310 ± 23 pg.mL^{-1}) but not significant ($p>0.05$).

TABLE 2. Brain and serum β-amyloid levels of rats in all groups (mean ± SEM)

Groups	Brain β-amyloid		Serum β-amyloid (pg. mL^{-1})
	Soluble (pg.mL^{-1})	Insoluble (pg.mL^{-1})	
CNT	326.45 ± 44.34	287.06 ± 18.26	436.91 ± 44.70
TRE1	508.50 ± 40.59$^{\#\#}$	323.73 ± 33.48$^{n.s}$	503.73 ± 130.49$^{n.s}$
TRE2	535.77 ± 36.24$^{\#\#}$	310.09 ± 23.11$^{n.s}$	250.09 ± 31.01$^{n.s}$

CNT: control group; TRE1: STZ 60 mg.kg^{-1} BW and LPS 3 mg.kg^{-1} BW; TRE2: STZ 30 mg.kg^{-1} BW and LPS 1.5 mg.kg^{-1} BW; ##: significant to CNT ($p<0.05$ ANOVA, Tuckey test); n.s.: not significant to CNT; n=4

Serum β-amyloid levels in each group of rats were measured following termination. The highest mean was in TRE1 group (504±130 pg.mL^{-1}) comparing with CNT (437±45 pg.mL^{-1}). Kruskal Wallis test showed that serum β-amyloid levels in TRE1 rats were not significant than those in CNT rats ($p>0.05$).

DISCUSSION

Memory dysfunction can be used as an early indicator of dementia. This condition can lead to an amnesic syndrome, a condition when an individual unable to consolidate and retrieve memory.[24] This failure causes the conditions of anterograde and retrograde amnesia. The type of memory that is affected in dementia is episodic memory, a subtype of declarative memory. Hippocampus and part of the temporal medial lobe are believed to play a major role in the formation of episodic memories, one of them is spatial memory. This function is conducted by the 'place cells' located in the CA1 and CA3 parts of the hippocampus.[25] To assess the ability to store and retrieve spatial memories, we used the MWM memory test. In addition, we also used the NOR test to assess the performance of another declarative memory i.e. recognition memory.

We used MWM to assess hippocampus plasticity without affected by external motivation like food. The escape latencies in the TRE1 rats were significantly longer than those in the CNT group ($p<0.05$), indicated the learning capacity was decreased after STZ and LPS treatment. Previous studies showed that AβOs disrupt excitatory synaptic function, suppress long-term potentiation, and facilitating long-term depression.[14] Moreover, in MWM probe trial showed consistent results where the durations in the target quadrant were lower in the treatment groups. The difference in sensorimotor performance can be neglected as we found no significant result in MWM visual cue test.

In addition to the MWM, the rats were subjected to a NOR task, that also

hippocampus-dependent learning tasks. Discrimination indexes of TRE1 and TRE2 rats were negative, it means that the rats spent less time exploring the novel object. Furthermore, ANOVA and Tuckey's test showed a significant result of DI in TRE1 and TRE2 rats than those in CNT rats, indicating that the rats could not remember the familiar object ($p<0.05$).

β-amyloid has a pivotal role in the pathobiology of AD. Both brain or other cells-derived β-amyloid fragment are created by the separation of Amyloid Precursor Protein (APP) initially after β-Site APP-cleaving enzyme 1 (BACE1) to produce C99 fragment and soluble APPβ, then the C99 fragment is separated by γ-secretase to yield β-amyloid.[26] Generally, there are two types of β-amyloid, insoluble β-amyloid fibrils (fAβ) and soluble oligomers (nonfibrillar) β-amyloid (AβO). Therefore, in the present research, we examine the soluble and insoluble form of β-amyloid after STZ and LPS administration. In the past decade, fAβ that constructs the extracellular plaques was proposed to be a major pathogenic factor of AD as amyloid cascade hypothesis.[27] However, recent findings in animal models and humans suggest that the accumulation of AβO have the same neurotoxic effects. These findings have established the AβO hypothesis.[14]

Our recent findings showed a significant increase in soluble brain β-amyloid, but no significant result in insoluble brain β-amyloid. Recent studies suggest that soluble β-amyloid, such as oligomers, ADDLs, globulomers, and protofibrils, may cause neurons injury.[28] Soluble β-amyloid enter neurons and initiate autophagy. Insufficient destruction of β-amyloid aggregates by autophagy may occur inside the neurons, resulting in lipofuscin accumulation that could worsen neuronal dysfunction.[29] An electrophysiological study showed that amyloid deposition lowering the long-term potentiation (LTP) in the hippocampus, both stimulation and preservation.[30] LTP weakness is correlated with low performance of individuals in memory tasks.

The results showed that IP STZ and LPS significantly increase soluble β-amyloid level. Since systemic STZ cannot penetrate blood-brain barrier due to lack of GLUT-2 receptors, STZ may influence brain metabolism indirectly, through alteration free fatty acid (FFA) level in blood or other substance that may toxic to the brain.[15] Meanwhile, LPS administration causes neuroinflammation, including acute (until day-7) soluble β-amyloid increase.[31] However, LPS alone did not hold β-amyloid at high levels in sub-acute response.[32] Therefore, our findings may be promising since β-amyloid increase was lasting until 30 days after single STZ and LPS injections.

We tried to elucidate the potential of chemical-induced animal models of dementia, which are more pertinent to the sporadic type of AD. Several pharmacological substances have also used to induce SDAT, they are ferrous amyloid buthionine, okadaic acid, Aluminium chloride, scopolamine, and trimethyltin.[33-35] Those substances were considered for the testing hypothesis of AD – cholinergic, tau protein, or amyloid. Our results suggested that STZ and LPS combination impaired memory performance and increase the soluble β-amyloid level as a hallmark of AD, which particularly tests the amyloid hypothesis of AD. Together with other chemical-induced models, a combination of STZ and LPS may participate in the current treatment development for AD. However, further molecular and electrophysiological research needs to be conducted to uncover the pathological mechanism of SDAT. Moreover, research using experimental animals is basic research that still has a long journey to be applied in humans.

CONCLUSION

The results showed that intraperitoneal injection of STZ (60 $mg.kg^{-1}$ BW) and LPS (3 $mg.kg^{-1}$ BW) indicate decrease in memory performance and increase soluble β-amyloid level in rats. These findings suggest that the treatment is prospective for SDAT non-transgenic animal model.

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REFERENCES

1. Chertkow H, Feldman HH, Jacova C, Massoud F. Definitions of dementia and predementia states in Alzheimer's disease and vascular cognitive impairment: Consensus from the Canadian conference on diagnosis of dementia. In: Alzheimer's Research and Therapy. Alzheimers Res Ther 2013; 5(Suppl 1):S2. https://doi.org/10.1186/alzrt198
2. Duong S, Patel T, Chang F. Dementia: what pharmacists need to know. Can Pharm J (Ott) 2017; 150(2):118-29. https://doi.org/10.1177/1715163517690745
3. Alzheimer's Association. 2019 Alzheimer's disease facts and figures. Alzheimers Dement 2109; 15(3):321-87.
4. Mc Khann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, *et al.* The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7(3):263–9. https://doi.org/10.1016/j/jalz.2011.03.005
5. Matthews BR. Memory dysfunction. Continuum (Minneap Minn) 2015; 21(3 Behavioral Neurology and Neuropsychiatry): 613-26. https://doi.org/10.1212/01/CON.0000466656.59413.29
6. Rogers TT, Ivanoiu A, Patterson K, Hodges JR. Semantic memory in Alzheimer's disease and the frontotemporal dementias: a longitudinal study of 236 patients. Neuropsychology 2006; 20(3):319–35. https://doi.org/10.1037/0894-4105.20.3.319
7. Pennanen C, Kivipelto M, Tuomainen S, Hartikainen P, Hänninen T, Laakso MP, *et al.* Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. Neurobiol Aging 2004; 25(3):303–10. https://doi.org/10.1016/S0197-4580(03)00084-8
8. El Haj M, Antoine P, Nandrino J-L, Kapogiannis D. Autobiographical memory decline in Alzheimer's disease. Ageing Res Rev 2016; 27:15-22. https://doi.org/10.1016/j.arr.2016.02.002
9. Bekris LM, Yu CE, Bird TD, Tsuang DW. Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol 2010; 23(4):213-27. https://doi.org/10.1177/0891988710383571
10. Lanoiselée HM, Nicolas G, Wallon D, Rovelet-Lecrux A, Lacour M, Rousseau S, *et al.* APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. PLoS Med 2017; 14(3):e1002270. http://doi.org/10.1371/journal.pmed.1002270

11. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol 2018; 25(1):59–70. https://doi.org/10.111/ene.13439
12. Raulin AC, Kraft L, Al-Hilaly YK, Xue WF, McGeehan JE, Atack JR, *et al.* The molecular basis for apolipoprotein E4 as the major risk factor for late-onset Alzheimer's disease. J Mol Biol 2019; 431(12):2248-65. https://doi.org/10.1016/*j*.jmb.2019.04.019.
13. Sanabria-Castro A, Alvarado-Echeverría I, Monge-Bonilla C. Molecular pathogenesis of Alzheimer's disease. Ann Neurosci 2017; 24(1):46-54. https://doi.org/10.1159/000464422
14. Ferreira ST, Lourenco M V., Oliveira MM, De Felice FG. Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. Front Cell Neurosci 2015; 9:191. https://doi.org.10.3389/fncel.2015.00191
15. Grieb P. Intracerebroventricular streptozotocin injections as a model of Alzheimer's disease: in search of a relevant mechanism. Mol Neurobiol 2016; 53(3):174152. https://doi.org/10.1007/s12035-015-9132-3
16. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001; 50(6):537-46.
17. Nahdi AMTA, John A, Raza H. Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in Rin-5F pancreatic β-cells. Oxid Med Cell Longev 2017; 2017:7054272 https://doi.org/10.1155/2017/7054272
18. Friederich M, Hansell P, Palm F. Diabetes, oxidative stress, nitric oxide and mitochondria function. Curr Diabetes 2009; 5(2):120-44. http://doi.org/10.2174/157339909788166800
19. Kahraman S, Aydin C, Elpek GO, Dirice E, Sanlioglu AD. Diabetes-resistant NOR mice are more severely affected by streptozotocin compared to the diabetes-prone NOD mice: correlations with liver and kidney GLUT2 expressions. J Diabetes Res 2015; 2015:450128 https://doi.org/10.1155/2015/450128
20. Arluison M, Quignon M, Nguyen P, Thorens B, Leloup C, Penicaud L. Distribution and anatomical localization of the glucose transporter 2 (GLUT2) in the adult rat brain - An immunohistochemical study. J Chem Neuroanat 2004; 28(3):117–36. https://doi.org/10.1016/j.jchemneu.2004.05.009
21. Vorhees CV, Williams MT. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006; 1(2):848–58. https://doi.org/10.1038/nprot.2006.116
22. Antunes M, Biala G. The novel object recognition memory: Neurobiology, test procedure, and its modifications Cogn Process 2012; 13:93-110. https://doi.org/10.1007/s10339-011-0430-z
23. Casali B, Landreth G. Aβ Extraction from murine brain homogenates. Bio Protoc 2016; 6(8):e1787 https://doi.org/10.21769/BioProtoc.1787
24. Kopelman MD. Disorders of memory. Brain 2002; 125(pt 10):2152–90. https://doi.org/10.1093/brain/awf229
25. Moser MB, Rowland DC, Moser EI. Place cells, grid cells, and memory. Cold Spring Harb Perspect Biol 2015; 7(2):a021808. https://doi.org/10.1101/cshperspect.a021808
26. Eteghad SS, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. Med Princ Pract 2015; 24(1):1–10. https://doi.org/10.1159/000369101
27. Hardy JA, Higgins GA. Alzheimer's

disease: the amyloid cascade hypothesis. Science 1992; 256(5054):184–5. https://doi.org/10.1126/science.1566067
28. Shankar GM, Walsh DM. Alzheimer's disease: Synaptic dysfunction and Aβ. Mol Neurodegener 2009;4:48. https://doi.org/10.1186/1750-1326-4-48
29. Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G. Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. Biochim Biophys Acta 2010; 1802(1):2-10. https://doi.org/10.1016/j.bbadis.2009.10.006
30. Korte M, Herrmann U, Zhang X, Draguhn A. The role of APP and APLP for synaptic transmission, plasticity, and network function: Lessons from genetic mouse models. Exp Brain Res 2012; 217(3-4):435-40. http://doi.org/10.1007/s00221-011-2894-6
31. Wang LM, Wu Q, Kirk RA, Horn KP, Ebada Salem AH, Hoffman JM, *et al*. Lipopolysaccharide endotoxemia induces amyloid-β and p-tau formation in the rat brain. Am J Nucl Med Mol Imaging 2018; 8(2):86–99.
32. Jendresen C, Digre A, Cui H, Zhang X, Vlodavsky I, Li JP, *et al*. Systemic LPS-induced Aβ-solubilization and clearance in AβPP-transgenic mice is diminished by heparanase overexpression. Sci Rep 2019; 9(1):4600. https://doi.org/10.1038/s41598-019-40999-4
33. Lecanu L, Papadopoulos V. Modeling Alzheimer's disease with non-transgenic rat models. Alzheimers Res Ther 2013; 5(3):17. https://doi.org/10.1186/alzrt171
34. Gilles C, Ertlé S, Macher JP. Pharmacological models in Alzheimer's disease research. Dialogues Clin Neurosci 2000; 2:247-55.
35. Nilsberth C, Kostyszyn B, Luthman J. Changes in APP, PS1 and other factors related to Alzheimer's disease pathophysiology after trimethyltin-induced brain lesion in the rat. Neurotox Res 2002; 4(7-8):625–36.