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RESEARCH ARTICLE

Neuroprotective Potency of *Tamarindus indica* Seed Extract for Preventing Memory Impairment in Rat Model of Alzheimer's Disease

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ABSTRACT:

Objective: To investigate the neuroprotective potency of *Tamarindus indica* seed extract (TSE) for Alzheimer's prevention-based on the pathogenesis of the disease. **Methods:** Rats were divided into 6 groups: normal, control, aluminum chloride ($AlCl_3$), low, moderate and high dose of TSE. The Morris water maze test was used to evaluate the memory. Proinflammatory cytokines levels were estimated by enzyme-linked immunosorbent assay method. Malondialdehyde levels were measured by the thiobarbituric acid reactive substances method. **Results:** Administration of 50 mg/kg TSE were decreased cerebral cortex levels of proinflammatory cytokines and lipid oxidation products, and protected memory impairment due to the induction of $AlCl_3$. **Conclusion:** Data from this study confirmed the neuroprotective benefits of TSE for Alzheimer's prevention based on the pathogenesis of the disease. Further, these results could lead to the development of Alzheimer's prevention.

KEYWORDS: Morris water maze, Aluminum chloride, IL1β, TNFα, Malondialdehyde.

INTRODUCTION:

Alzheimer's is a progressive-neurodegenerative disorder, characterized by a decline in cognitive function, mood changes and behavior[1,2]. Alzheimer's is the most common form of dementia, reaching 60%-70%[3]. Memory impairment is a major clinical symptom in Alzheimer's cases[4]. Several hypotheses about the pathogenesis of Alzheimer's disease have been revealed including neuroinflammation, oxidative stress, beta-amyloid and hyperphosphorylation of tau proteins[5-8]. Current Alzheimer's pharmacotherapy from the cholinesterase inhibitor and N-methyl-D-aspartate antagonist groups has not produced results as expected[9,10]. Based on this fact, research is still ongoing to invent the potential of various herbal ingredients for the treatment and prevention of Alzheimer's[11-13].

Aluminum chloride ($AlCl_3$) has been used as an induction in rats to produce animal models of Alzheimer's[14,15]. Administration of $AlCl_3$ in the rat can trigger inflammation and oxidative stress in the brain, memory decline and neuropathological changes similar to Alzheimer's in the form of senile plaques in the hippocampus and cortex areas[16,17].

Tamarindus indica is an endemic plant in the tropics. *Tamarindus indica* seeds are round or rhomboid, flattened with a shiny and hard seed coat, red to light brown in color, in which there are cotyledons[18]. In traditional medicine, *Tamarindus indica* seeds have been widely used as therapy in various diseases including tumors, gastric ulcers and antidiabetic[19,20]. Research Chunglok et al[21] explained that *Tamarindus indica* seeds contain higher polyphenol compounds and antioxidant effects than other plant seeds. The polyphenol compounds in plants are believed to provide neuroprotective benefits through anti-inflammatory and antioxidant effects[22,23]. Towards this study, we aim to show the neuroprotective potency of *Tamarindus indica*

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seed extract (TSE) for the prevention of Alzheimer's in a rat model based on the pathogenesis of the disease.

MATERIALS AND METHODS:

Chemicals:

Aluminum chloride and thiobarbituric acid were procured from Tokyo Chemical Industry, Portland, USA. ELISA kits for IL1β and TNFα were procured from Bioassay Technology Laboratory, Shanghai, China. All other chemicals used were analytical grade.

Animals and treatment:

Wistar rats aged 3 months with 200grams of weight were used for this research. The rats were kept in individual cages, with a 12-hour cycle of light and given food and drink in ad libitum. The rats were divided randomly into 6 groups (n = 5). Group I rats were not given any treatment. Group II rats were orally administered distillate water and saline for 10 weeks. Group III rats were orally administered $AlCl_3$ (300 mg/kg/day) for 10 weeks. Group IV rats were orally administered $AlCl_3$ (300mg/kg/day) and TSE (25 mg/kg/day) for 10 weeks. Group V rats were orally administered $AlCl_3$ (300mg/kg/day) and TSE (50 mg/kg/day) for 10 weeks. Group VI rats were orally administered $AlCl_3$ (300mg/kg/day) and TSE (100 mg/kg/day) for 10 weeks. Five grams of $AlCl_3$ was dissolved in 100mL of distilled water[24]. The rats were weighed every week to make dosage adjustments. Ten weeks after oral treatment, the Morris water maze test was carried out. Next, the rats were anesthetized by using ether, then sacrificed by cervical dislocation and took their brain. And the levels of IL1β, TNFα, and malondialdehyde (MDA) cerebral cortex were estimated. The animal experiment was reviewed and approved by the Ethical Committee of Faculty of Medicine, University of Jember (Letter No. 1151/H25111/KE/2017).

Preparation of TSE solution:

Five grams of TSE was dissolved in 100mL of saline[25]. *Tamarindus indica* seeds were obtained from the trees in the Jember region. One hundred grams of *Tamarindus indica* seed powder was soaked in 500mL of methanol for 3 days. The levels of three polyphenols were measured using HPLC-MS, the concentration of procyanidin B2 was the highest, then myricetin and the lowest concentration was caffeic acid, the results have been published[26].

Morris water maze:

The Morris water maze test was used to evaluate the spatial memory of rats. The test was performed according to the previously described method[27]. A black circular swimming pool (diameter of 150cm, depth of 50cm) was filled with a water height of 30cm. The inner wall pool above the water surface was placed spatial cues and on the room wall test was placed spatial cues too. A circular platform (diameter of 10cm) was placed in the middle of one of the pool quadrants. Recorded the time of the rats spent to reach the platform (time escape latency). The maze test was carried out for 5 consecutive days with two repetitions each day.

Assessment of IL1β and TNFα:

The cerebral cortex levels of IL1β and TNFα were estimated quantitatively by using ELISA kits as per the manufacturer's protocol.

Assessment of MDA:

The cerebral cortex was weighed and homogenized in ice-cold medium containing 50mM Tris/HCl and 300 mM sucrose at pH 7,4 at a ratio of 10% (w/v). Then it was centrifuged 1400g for 10 minutes at 4°C. The supernatant was stored at -80°C[28]. MDA was assessed as per Sinha et al[29]. Briefly, the supernatant (0,25mL) was added to 0,25mL of 20% trichloroacetic acid, next centrifuged at 1000rpm for 4 minutes. Thiobarbituric acid (0,5mL 0,67% in 0,026M tris buffer) was added to the supernatant and heated at 100°C for 15-30 minutes. It was cooled in ice for 10 minutes, and the absorbance was read at 532nm using a Thermo Spectoric spectrophotometer.

Statistical analysis:

All result data were presented as mean±standard deviation. To assess the differences between the groups, one way ANOVA was performed and continued by Tukey's HSD post hoc analysis. A value of $p<0,05$ was considered to be significant.

RESULTS:

Morris water maze:

In all groups there was a learning process during 5 days of Morris water maze test, it was indicated by the shorter time escape latency. The $AlCl_3$ group as an Alzheimer's model demonstrated significant long time escape latency compared to the normal group on days 2,3 and 4 of the Morris water maze test ($p<0,05$), and it was indicated the impairment on spatial memory. The preventive potency of TSE (50mg/kg/day) to Alzheimer's showed significant protection of memory impairment on days 2 and 3 of the Morris water maze test compared to the $AlCl_3$ group ($p<0,05$) (Figure 1).

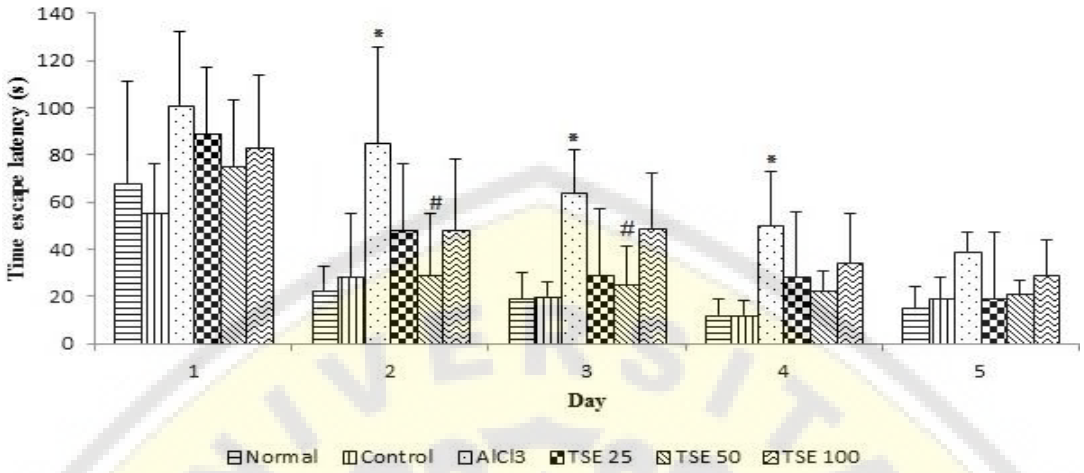
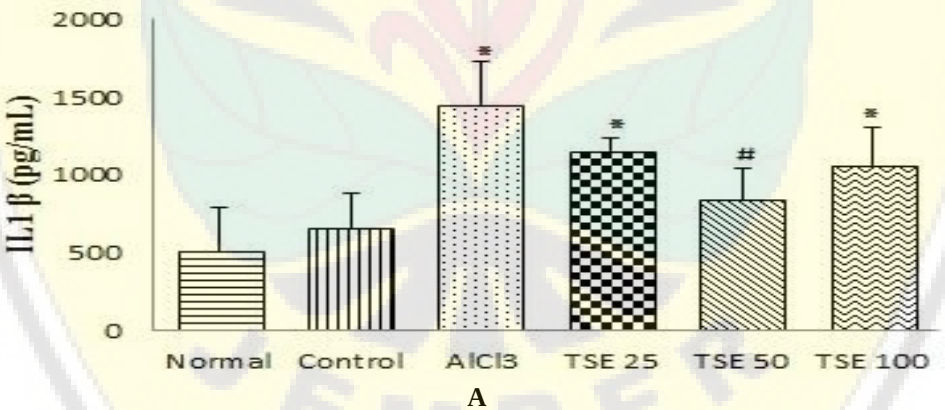


Figure 1. Morris water maze.
Data shown are mean groups ± SD. *compared to the normal group p<0.05. #compared to $AlCl_3$ group p<0.05.

ELISA IL1β and TNFα:

The result of the ELISA assay to the cerebral cortex levels of IL1β and TNFα showed in figure 2. The cerebral cortex levels of proinflammatory cytokine IL1β and TNFα were a significant elevation in the $AlCl_3$ group compared to the normal group (p<0,05). The protective effect for Alzheimer's as an anti-inflammatory from TSE at dose 50 mg/kg/day appears to restore the levels of IL1β and TNFα to a normal level significantly in comparison with the $AlCl_3$ group (p<0,05), and it has not appeared in another dose of extract.



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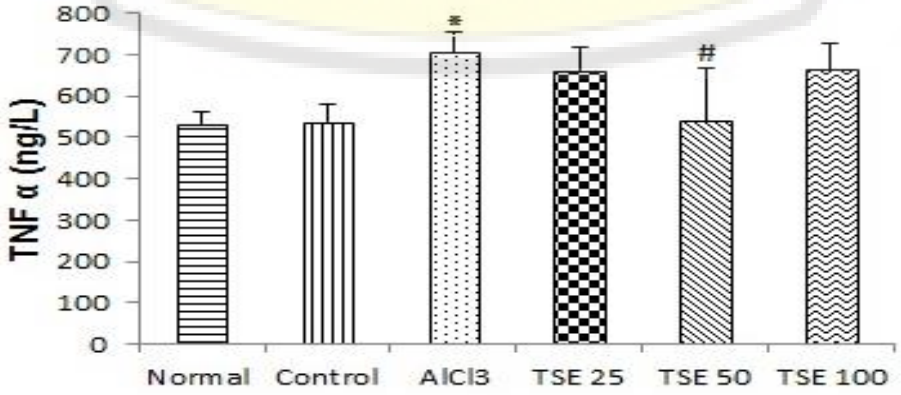

B

Figure 2. ELISA assay for the cerebral cortex of proinflammatory cytokines. (A) IL1β, (B) TNFα.
Data shown are mean groups ± SD. *compared to the normal group p<0.05. #compared to $AlCl_3$ group p<0.05.

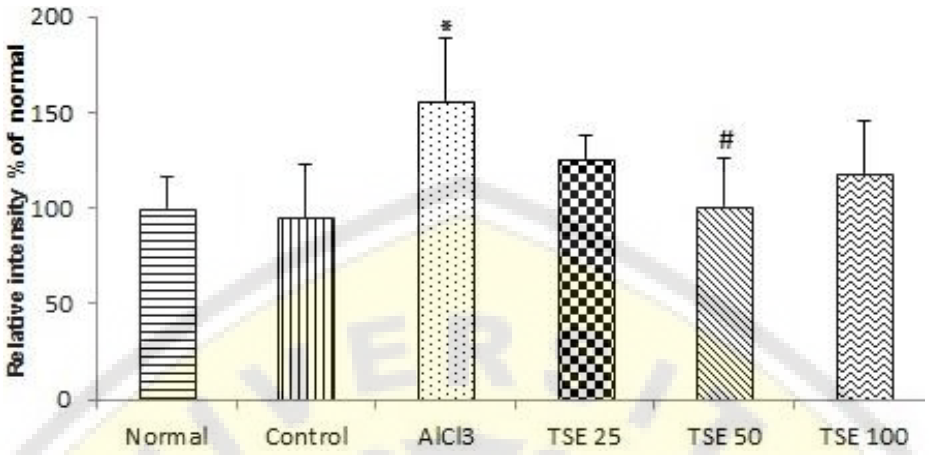

Figure 3. MDA cerebral cortex levels.
Data shown are mean ± SD groups. *compared to the normal group p <0.05. #compared to $AlCl_3$ group p <0.05.

MDA levels:

The levels of cerebral cortex MDA in the research showed in figure 3. MDA levels as the product from lipid oxidation were significantly elevated in model Alzheimer's group with $AlCl_3$ induction compared to the normal group (p<0,05). The neuroprotective potency from TSE appeared in the lower of MDA cerebral cortex levels. The MDA levels of TSE group (50 mg/kg/day) was significantly reduced in comparison with the $AlCl_3$ group (p<0,05).

DISCUSSION:

Orally administration $AlCl_3$ to rats can penetrate the blood-brain barrier and can accumulate in brain tissue[14]. Exposure to $AlCl_3$ can cause an increase in the concentration of glial fibrillary acidic protein and ionized calcium-binding adapter molecule 1, it indicates the activation of microglia and astrocytes due to the induction of $AlCl_3$[30]. Activation of microglia and astrocytes will cause the release of proinflammatory cytokines such as IL1β and TNFα[31,32]. One of the effects of aluminum exposure on brain tissue is that it would accelerate the reaction of Fe in producing free radicals, then free radicals may cause an increase in lipid oxidation[33]. The outcome of lipid peroxidation is malondialdehyde[34]. Our results indicate that the administration of $AlCl_3$ causes a significant increase in cerebral cortex levels of IL1β, TNFα, and MDA. The results of this study are in line with other studies of oral administration $AlCl_3$ 100 mg/kg to albino rats for 6 weeks resulting in an increase in TNFα proinflammatory cytokines in the hippocampus and cortex areas[16]. The oral administration $AlCl_3$ in Wistar rats at a dose of 500 mg/kg for one-month results in an increase in MDA level in the hippocampus and cortex area[17].

On the other hand, the results of our study showed that the oral administration of $AlCl_3$ caused memory impairment. Other research explains that the occurrence of memory disorders can occur through inflammation and oxidative stress. Intra-cerebral IL1β injection results in spatial memory deficits due to a disturbance in noradrenergic neurotransmitters and serotonin[35]. TNFα injection into rat brain can generate interference with long term potentiation and a decrease in the number of cholinergic neurons[36,37]. An excessive amount of reactive oxygen species can excessively stimulate N-methyl-D-aspartate receptor (NMDAR), disruption of the synaptic plasticity and continues with impaired memory[38,39]. Our study is bolstered by the research showing that the administration of $AlCl_3$ orally through drinking water at a dose of 100 mg/kg for 6 weeks in Sprague Dawley rats causing a decrease in memory by Morris water maze test[14].

Two Alzheimer's pharmacotherapies' groups such as the cholinesterase inhibitor and NMDA antagonists was felt to be more symptomatic. Other therapies that were being developed such as immunotherapy, antioxidant and β secretase inhibitor groups have not given maximum results[9,10]. Preventive efforts become mainly needed in Alzheimer's disease. Consumption of vitamin E from food and diet with a Mediterranean diet (a diet high intake of fish, vegetables, nuts, unsaturated fats and instead of low meat) can reduce the risk of Alzheimer's[40,41]. This research is exploring the benefits of TSE for the prevention of Alzheimer's based on the pathogenesis of diseases in rat models. Our identified results of the polyphenol TSE content are in line with the analysis conducted by Razali et al[42] with UHPLC obtained the content of three compounds with the highest concentrations of procyanidin B2, caffeic acid, and myricetin. Based on computer modeling, the three compounds have strong anti-inflammatory and antioxidant potential[26]. TSE has anti-inflammatory potency in the asthmatic model[43]. Research conducted by Sundaram et al[25] confirms that there is the anti-inflammatory and antioxidant potential from TSE dose

of 50mg/kg/day orally administered in rats with arthritis for 2 weeks.

Research on the effects of each polyphenol contained in the TSE has been done on various organ systems and other diseases. The oral administration caffeic acid in DM rat model resulted in a decrease of IL1β, IL6, and TNFα levels as well as a reduction in the expression of IL1β, IL6 and TNFα mRNA in the heart tissue of rat[44]. In the animal study, caffeic acid has antioxidant potency to influence glucose metabolism[45]. *In vivo* research using the ulcerative colitis rat model, an oral administration myricetin confirms the decreased of IL1β and IL6 levels in colon tissue[46]. Oral treatment of procyanidin B2 in CCl4-induced rats shown a reduction of MDA levels and an increase in superoxide dismutase, catalase and glutathione peroxidase enzymes in the liver tissue[47]. The results of our current research indicate that the administration of TSE is able to normalize IL1β, TNFα, MDA cerebral cortex levels and to prevent memory impairment in Alzheimer's rat model. More intensive research is still needed to assess the advanced benefits from the utilization of TSE in various biomarkers and Alzheimer's model before clinical trials.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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