

Research Article

The Differences Between the Effect of Oral and Intraperitoneal Induction of Aluminum Chloride (AlCl₃) on the Memory Function of White Rats (*Rattus norvegicus* strain wistar) Menopause Model

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The decline in estrogen at menopause leads to a loss of neuroprotective function. One of the effects is Alzheimer's disease (AD), with clinical manifestations of dementia, decreased memory function, language, thinking, and learning. The pathology of AD is widely studied but not completely understood. Various animal models have been used but have not been able to represent the pathology of AD fully. This study aimed to determine the effect of oral and intraperitoneal induction of aluminium chloride (AlCl₃) on the memory function of menopausal model white rats (*Rattus norvegicus* strain wistar). This study used 16 female rats, divided into four groups, namely the negative control group (K1), the ovariectomy group (OVX, K2), and the OVX + AlCl₃.6H₂O group 100 mg/kg BW orally (K3), and the OVX + AlCl₃.6H₂O group. 70 mg/kg body weight intraperitoneally. AlCl₃.6H₂O induction was given for six weeks. In the last five days, the Morris water maze test was carried out. From the MANOVA test, the p-value of F values in Pillai's trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root were < 0.05. Based on the post hoc Bonferroni test on the fifth day of the trial, there was a significant difference between the standard and OVX groups with the AlCl₃ induction group, and there was no significant difference between the oral and intraperitoneal induction groups. Conclusion: There is no significant effect of oral and intraperitoneal induction of Aluminum Chloride (AlCl₃) on the memory function of white rats (*Rattus norvegicus* strain wistar) menopausal model.

Keywords: menopause, Alzheimer's disease, memory test

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease with clinical features of decreased memory and cognitive or dementia accompanied by reduced function of memory, thinking, language, and learning capacity. (1,2) The etiology of AD is not

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known with certainty and the main risk factor is age. The majority of patients found a combination of genetic and environmental factors. (3)

Women who experience menopause will cause a loss of estrogen function, one of which is neuroprotective. (4–6) Several hypotheses state that the etiology of AD is metal intoxication (one of which is aluminum), impaired immunity, air pollution, infection by viruses, neurotransmitter disorders, trauma, and hereditary predisposition. (7) Aluminum has been known as a neurotoxin (8){Bibliography} that causes inhibition of long-term potentiation (LTP), triggers an inflammatory response, affects the speed of transport in axons, and causes abnormal synaptic structure, resulting in memory loss. (3)

Several studies were conducted by giving AlCl₃ (aluminum chloride) in various doses to male rats via oral and intraperitoneal routes, which influence memory function. (9–11) While Andriana et al. (2021) reported no difference in memory function in ovariectomized rats given oral AlCl₃. This study was conducted to compare the memory function of ovariectomized rats (menopausal model) given oral and intraperitoneal AlCl₃.

2. MATERIALS AND METHODS

This research is a True Experimental study with the post-test only control group design experiment, conducted in the Pharmacology laboratory of the Faculty of the Medicine University of Muhammadiyah Malang for 6 (six months). The samples were female rats (*Rattus norvegicus* strain wistar) aged eight to ten months, with body weight ranging from 185-200 grams and in good health, indicated by their active movements. Experimental animals were placed in individual cages. They were provided with drinking water *ad libitum* and standard feed. The cages were placed at room temperature, adjusted humidity, and regular circulation with light and dark periods of 12 hours each. At the initial stage, one week of adaptation was carried out on the sample. Then an ovariectomy was performed using the modified Ingle DJ and Griffith JQ, 1971 method, and a second adaptation was carried out for two weeks to obtain hypoestrogenic conditions. A total of 16 samples were divided into four groups (12) namely 1 negative control group (K1), ovariectomy group (OVX, K2), OVX+AlCl₃.6H₂O 100 mg/kg body weight orally (K3) and OVX +AlCl₃.6H₂O 70 mg/kg BW intraperitoneal (K4). AlCl₃.6H₂O induction was given for six weeks. The Morris water maze test was performed on days 38-42 of induction.

The research was conducted after obtaining approval from the Health Research Ethics Committee University of Muhammadiyah Malang with the reference number E.5.a/145/KEPK-UMM/VIII/2019.

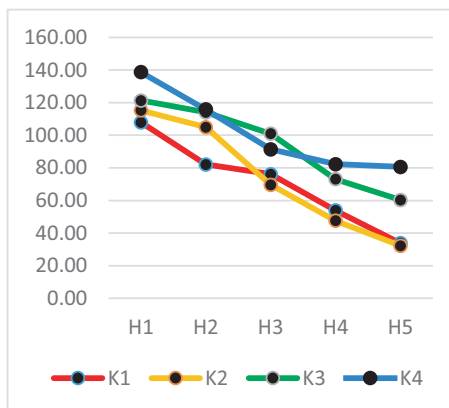
3. RESULTS

In this study, there were improvements in memory tests in all groups.

TABLE 1: Morris Water Maze (MWM) test results.

Group	Average Time Required (seconds)					p value
	Observation on days					
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	
K1	107.90 ± 14.92	82.06 ± 12.24	79.17 ± 9.62	53.87 ± 9.79	33.81 ± 4.65	0.000 - 0.002
K2	115.25 ± 11.93	105.00 ± 9.42	72.12 ± 16,20	47.46 ± 12,89	32.23 ± 10.99	
K3	121.35 ± 13,90	114,12 ± 14.21	100,96 ± 23.47	72.08 ± 39.84	60.36 ± 16.04	
K4	138.75 ± 17.77	115.85 ± 14.00	91.42 ± 0.66	82.25 ± 11.66	80.56 ± 12.58	

Description : K1 : negative controle, K2 : OVX; K3 : OVX+AICI3.6H2O 100 mg/kg BW peroral; K4 : OVX+AICI3.6H2O 70 mg/kg BW intraperitoneal. P-value : Pillai's trace : 0.002, Wilks' Lambda : 0.001, Hotelling'a Trace : 0.001 dan Roy's Largest Root : 0.000



Description : K1 : negative controle, K2 : OVX; K3 : OVX+AICI3.6H2O 100 mg/kg BW peroral; K4 : OVX+AICI3.6H2O 70 mg/kg BW intraperitoneal

Figure 1: Morris Water Maze (MWM) test results.

Based on the MANOVA test, each p-value of the F value in Pillai's trace: 0.002, Wilks' Lambda: 0.001, Hotelling'a Trace: 0.001, and Roy's Largest Root: 0.000. This shows that there was a significant difference in the results of the memory test due to the treatment of the sample. Based on the post hoc Bonferroni test, there was a significant difference between the negative control and OVX with the AICI3 induction group, and there was no significant difference between the oral and intraperitoneal AICI3 induction.

4. DISCUSSION

Various experimental animal models from primate and non-primate groups were used in the study. However, they are still not satisfied because they have not been able

to display the overall changes in AD pathology. (13) Most AD cases are sporadic (sAD), and the underlying cause of these cases is still unknown. Neuropathologically, AD is characterized by the accumulation of amyloid plaques (Ab) and NFT, extensive synaptic loss, inflammation, oxidative stress, and neuronal degeneration. It is clinically and neuropathologically indistinguishable between familial AD and the sporadic, young-onset form of familial AD. (14)

More than 150 clinical trials failed to improve cognitive decline and AD pathology. This is due to an over-reliance on inadequate animal models and partly because the drugs are targeted at treating only the symptoms and not the underlying cause.(15)

The etiology of AD is unknown, using experimental animal models utilizing genetic mutations associated with familial AD (FAD). This genetic model is advantageous in determining disease progression's molecular mechanisms and testing potential therapies. Although no single mouse model recapitulates all aspects of the disease spectrum, each model allows for an in-depth analysis of one or two disease components. (14)

In another study, Wistar rats were used, which had a life span of 24 months. Using the polynomial model, mice less than six months old are identical to humans less than 20 years old. The middle age of mice is 12-18 months, similar to that of humans under 40 years old. Rats over 24 months old are equivalent to humans over 60 years old. Cognitive and neurobiological changes have been observed in middle-aged mice and become more pronounced with age. These processes occur in elderly humans. The use of non-transgenic mice has limitations because AD has no pathological features such as AB and NFT. (13) On the other hand, the use of transgenic mice shows more familial AD than sporadic AD, even though more than 95% of AD features are sporadic or late onset. (16)

Ali et al. (2016) conducted a study on 80 male Sprague Dawley rats given aluminum chloride – hydrated ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) via intraperitoneal (i.p) injection for six weeks with doses of 50, 70, and 100 mg/kg body weight. This study obtained a decrease in learning ability and memory. (9)

Rather et al. (2018) conducted a study on male white Wistar rats weighing 200-225 g which were induced with AlCl_3 100 mg/kg BW orally for six weeks. The results showed increased AI levels and AChE activity, decreased memory and learning ability (with the MWZ test and passive avoidance test), increased expression of APP, $\text{A}\beta$ 1-42, beta and gamma-secretase, glial fibrillary acidic protein, ionized calcium-binding adapter molecule 1, IL-1 β , IL-6, IL-4, IL-2, TNF, iNOS, NF- and COX-2 in the hippocampus and cerebral cortex.(3)

5. CONCLUSION

There was a significant difference in the memory test results due to the sample's treatment. There was no difference in the effect of oral and intraperitoneal aluminum chloride (AlCl₃) induction on memory function of menopausal model rats (*Rattus norvegicus* strain wistar).

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