Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Biochemical effect of aluminum chloride induced brain damage in mice

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ARTICLE INFO

Keywords

ALCL₃

Amyloid beta (Aβ)

Mice

Neurotoxicity

Oxidative stress

Received 12/01/2024
Accepted 21/03/2024
Available On-Line 01/04/2024

ABSTRACT

Neurotoxicity is commonly associated with the central and/or peripheral nerve systems structural damage. It happens when a chemical interferes with the normal functioning of the nervous system, potentially resulting in the death of neurons transmitting and information-processing. The purpose of this study was to investigate the effect of aluminum chloride ($AlCl_3$) induced neurotoxicity in mice. Forty mice were allocated into two equal groups. Group 1 (Normal control), which fed a regular laboratory diet for four weeks. Group 2 (Aluminum chloride) rats received $AlCl_3$ orally at a dose of (8.5 mg/kg/day) for four weeks. The obtained results revealed a significant increase in brain tissue amyloid beta, nitric oxide and in serum cortisol, complement C3 and C4 concentrations, while brain tissue SOD and CAT activities were markedly decreased in $AlCl_3$ treated mice as compared with normal control. In conclusion, $AlCl_3$ has toxic and harmful effects with noticeable oxidative stress and inflammation.

1. INTRODUCTION

Brain impairment is the most devasting neurodegenerative disorders, accounting for over 80% of dementia cases globally which cause progressive loss of cognitive functions, particularly memory, that limits the ability of patients to perform everyday activities and impairs occupational or social functions (Sabogal-Guaqueta et al., 2020; Tyagi and Pugazhenthi, 2021).

The most common type of dementia is brain dysfunction. Its pathophysiology is complex and involves the buildup of pTau (tau protein) with subsequent development of NFTs, deposition of Aβ plaques (amyloid beta) and neurodegeneration (Sabogal-Guaqueta ´ et al., 2020). Senile plaques, neurofibrillary tangles, neuro-inflammation contributing to neuronal degeneration are hallmark features of brain impairment, (Sabogal-Guaqueta et al., 2020).

However, genetic predisposition to brain damage is assumed to be predominantly connected to the apolipoprotein E (Apo E) genotype; also, ageing is a major risk factor for cognitive decline. Numerous other potential environmental factors are correlated with brain impairment including coronary heart disease, hypercholesterolemia, atherosclerosis, smoking, obesity and diabetes (Armstrong et al., 2019).

Trace elements and certain metals, such as aluminum (Al), mercury, copper, arsenic, lead, and manganese, are also poisonous in high concentrations (Abd-Elhady et al., 2013). Aluminum (Al) is neurotoxic, and its oral intake buildups in tissues, e.g., bones, muscles, and kidney results in a variety of neurological problems, particularly brain impairment. Aluminum produces increased amyloid buildup and has been linked to frontal brain disruption. Furthermore, Al_3 has been discovered in individuals with brain damage (Walton, 2014). Moreover, Aluminum is classified as a neurotoxic since it has harmful effects on brain development either pre- or post-natal (Dórea, (2015). Long-term consumption promotes neuroinflammation and cognitive function impairments. Neuroinflammation changes the density of dendritic spines, which impacts cognitive performance (Cao et al., 2016). Aluminum may pass the blood-brain barrier accumulate in brain, and hippocampus having the greatest quantities (Li et al., 2015). Aluminum buildup in the hippocampus leads to cognitive impairment primarily by inhibiting long-term potentiation via the glutamate nitric oxide-cyclic guanosine monophosphate pathway (Prakash et al., 2013). Our study aimed to investigate the adverse effect of aluminum chloride on brain tissue of hippocampus and cortex.

2. MATERIAL AND METHODS

The Ethical Animal Committee of Benha University, Faculty of Veterinary Medicine, accepted all experiment's protocol for the care of the laboratory animals (BUFVTM 05-12-23).

2.1. Chemicals

Aluminum Chloride ($AlCl_3$) was purchased from Sigma-Aldrich, St. Louis, Mo, USA.

2.2. Animals

The animal house at Benha University, Faculty of Veterinary Medicine, Egypt provided forty male mice (20-30 g). Before the trial, they were acclimatized for one week at the biochemistry department's animal facility under controlled environmental conditions. Fresh food and tap water were available on a regular basis.

2.3. Experimental design:

Mice were randomly divided into two groups (20 mice/each) placed in individual cages as follow:

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Group 1: (Normal control) mice were given oral saline only. Group 2: ($AlCl_3$) Mice received 8.5 mg/kg b. wt /day orally for four weeks to induce neurotoxicity (Amjad , 2015).

2.5. Sampling

Blood samples and brain tissue specimens were obtained from all animal groups twice at 2 and 4 weeks of the experiment.

2.5.1. Blood samples

Blood samples for serum separation were obtained from the retro-orbital venous plexus in clean dry tubes, centrifuged at 3000 rpm for 15 minutes, and the serum was kept at -20 °C for subsequent biochemical analysis. All sera were analyzed for determination of cortisol, complement C3 and C4**.**

2.5.2. Tissue samples

Ten mice from each group were rats were scarified by cervical decapitation, and the brain was quickly excised and cleaned to remove any blood or clots, then placed between two filter papers and stored at -20 °C for further analysis.

2.6. Preparation of brain homogenate

Brain tissues were homogenized in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 5000 rpm for 5 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: Amyloid beta (Aβ), nitric oxide (NO), superoxide dismutase (SOD) and catalase (CAT).

2.7. Biochemical Analysis:

Serum cortisol was determined according to the method described by Munro and Lasley, (1988). Complement C3 and C4 were determined using ELISA kit (Abbot Biomedical Company, Cot No. KT-30-3965) according to the manufacturer's instruction respectively. Moreover, brain tissue Amyloid beta (Aβ), nitric oxide (NO), superoxide dismutase (SOD) and catalase (CAT) were determined according to the method described by Mouse Beta Amyloid 1-42 (Sandwich ELISA) ELISA Kit - LS-F23031, Montgomery and Dymock, 1961), Misra and Fridovich (1972) and Clairborne (1985), respectively.

2.8. Statistical Analysis

All data were presented as SEM. The student's *t*-test was used for statistical analysis (Steel and Torri et al., 1980).

3. RESULTS

The obtained data presented in tables (1-3) showed that Aluminum chloride intoxicated mice show a significant increase ($P < 0.001$). in brain tissue Amyloid beta (Aß) and NO, and a significant decrease in brain tissue SOD and CAT activities. Conversely, serum cortisol, complement C3 and C4 concentrations were significantly increased in $AlCl_3$ exposed mice as compared with control group.

Table 1 Effect of $AlCl_3$ exposed mice on brain tissue Amyloid beta (Aß), Nitric oxide (NO) and serum Cortisol concentrations.

Animal groups	Amyloid beta (U/g)		Cortisol (ng/ml)		Nitric oxide (mmol/l)	
	2 Weeks	4 Weeks	2 Weeks	4 Weeks	2 Weeks	4 Weeks
G1:(Normal Control).	3.14±0.14	3.18±0.14	3.00±0.13	3.11±0.16	2.62±0.12	2.65±0.10
G2: ($AlCl_3$).	10.52±0.44***	19.70±0.49***	11.63±0.66***	25.17±1.37***	5.97±0.08***	12.34±0.60***

Data are presented as (Mean ± S.E). S.E = Standard error. *** Very highly Significant at ($P < 0.001$).

Table 2 Effect of $AlCl_3$ exposed mice on brain tissue CAT and SOD activities.

Animal groups	Catalase (U/ min/ gm		Superoxide dismutase (U/g)	
	2 Weeks	4 Weeks	2 Weeks	4 Weeks
G1:(Normal Control).	17.71±0.51	17.78±0.34	51.87±1.72	51.20±2.00
G2: ($AlCl_3$).	7.32±0.28***	4.08±0.31***	15.30±0.47***	7.66±0.45***

Data are presented as (Mean ± S.E). S.E = Standard error. *** Very highly Significant at ($P < 0.001$).

Table 3 Effect of $AlCl_3$ exposed mice on serum Complement 3 (C3) and Complement 4 (C4) concentrations.

Animal groups	Complement 3 (mg/d)		Complement 4 (mg/dl)	
	2 Weeks	4 Weeks	2 Weeks	4 Weeks
G1:(Normal Control).	82.43±2.96	81.63±3.15	41.37±1.65	40.87±1.85
G2: ($AlCl_3$).	173.30±3.16***	208.27±5.04***	73.60±2.76**	102.73±1.79***

Data are presented as (Mean ± S.E). S.E = Standard error. ** Highly Significant at ($P < 0.01$). *** Very highly Significant at ($P < 0.001$).

4. DISCUSSION

In the current study $AlCl_3$ intoxicated mice showed a significant increase in brain tissue Amyloid beta (Aß) and NO, and a significant decrease in brain tissue SOD and CAT activities. Conversely, serum cortisol, complement C3 and C4 concentrations were significantly increased in $AlCl_3$ exposed mice as compared with control group. These findings were essentially identical to those of Doungue et al., (2018), who found that aluminum-treated rats (32.5 mg/kg b.wt) for 60 days had a substantial decreases in CAT and SOD activities in the hippocampus and cortex compared to control. The decrease in SOD and CAT activities in brain homogenate following treatment with $AlCl_3$ was mostly attributable to decreased enzyme protein synthesis as a result of greater intracellular aluminum concentrations (Elhadidy et al., 2018). Furthermore, Chen et al. (2019) found that $AlCl_3$ causes a considerable rise in NO levels in the brain hippocampus and cortex.

Oxidative stress is described as an increase in the formation of ROS that are not eliminated adequately owing to compromised anti-oxidative systems, resulting in progressive organ failure (Salem et al., 2018). The current considerable rise in NO in the cortex, hippocampus, and striatum might be attributed to aluminum-induced NO synthase amplification (Czechowska et al., 2015). When NO reacts with ROS such as superoxide, peroxy nitrite is formed, which is one of the most toxic chemicals to the nervous system. This might be one of the processes behind aluminium-induced neurotoxicity (Poderoso, 2009).

This study indicated a significant rise in serum cortisol level in $AlCl_3$ treated mice which agree with Vasanthan and Joshi (2018), who observed that cortisol level was considerably higher in rats injected with $AlCl_3$ (320 mg/kg body weight) for 30 days compared to the control group. The cortisol level is widely established as a measure of stress severity (Sandstrom, 2005).

The existing results revealed a significant increase in the level of brain Amyloid beta (Aß), which is consistent with the findings of Yang et al. (2019) and McDonald et al. (2021), who found that aluminium treatment enhanced the levels of AI -42 (Amyloid I-42) in the hippocampus. Other research has shown that aluminium can affect a structure and sheet structure content, implying that it simplifies A peptide

aggregation (Zhang et al. 2019). Furthermore, aluminium accumulates in the hippocampus and frontal cortex, resulting in increased APP expression (amyloid proteins particles) and A deposition (Abdel-Aal et al., 2011). Aisen et al. (2017) identified a deposition as a major neuropathological characteristic and a crucial beginning event in the pathogenesis of brain damage. Because the hippocampus is involved in short-term memory processing, the formation of A plaques is critical; the recruitment of neurotoxic A peptides lead to disruption in synaptic dysfunction and homeostasis, with astrocytes and microglia hyper-activation (Shastrietal., 2013; Aisen et al., 2017). Additionally, A insults and failure to clear causing increasing of A1-42 peptides, which bind to AMPA receptors and Ca^{+2} channels, resulting in elevation of intracellular Ca^{2+}, resulting in chronic neuroinflammation and ROS production and complement proteins via microglia over time. Importantly, continued exposure to $AlCl_3$ causes the formation of A aggregates as well as significant oxidative damage (Rather et al. 2019; Promyo et al. 2020).

Microglia are immunological cells that live in the brain. They may react fast to a variety of danger signals and play critical functions in inflammation and cell debris removal. Activation of microglia led to the clearance of hyper-produced A and offered early protection against the disease through synthesis and release of anti-inflammatory cytokines in brain damage, most likely at the prodromal stage of disease (Cuello, 2017; Merlo et al., 2020). However, after long aluminium exposure, microglia's protective activity waned, and their overactivation resulted in changes in their gene expression leading to production of pro-inflammatory cytokines, neuro-inflammation, oxidative stress and A-associated neuronal damage amplification (Zhang G. et al., 2021).

Furthermore, the obtained findings are consistent with those of Fromell et al. (2020), who found higher C1q, C3, and C4 co-localization levels with A plaques in brain tissues from patients with cognitive impairment. Another research found higher C3 and C4 levels in the temporal cortex of patients with cognitive damage (Schartz et al., 2020).

Complement failure is believed to be contributing to neuroinflammation and subsequent neurodegeneration in a person with brain impairment decades before clinical symptoms emerge; this might be owing to A buildup, which overwhelms the complement system and drives the pathology of Alzheimer's disease. Kishore et al., (2003) discovered that A1-42 may activate the classical pathway directly by binding to C1q via its globular domain. C1q may bind to tau via the C1qA collagen domain and activate the classical pathway, according to Yang et al. (2000). C1q binding to A and tau may thus contribute to complement activation and neurodegeneration in persons with brain injury. Because of the BBB, the CNS was thought to be immune-privileged. However, it is now acknowledged that astrocytes, microglia, and neurons inside the CNS may produce complement components (Shastri, et al., 2013). The complement system may be both neuroprotective and neurotoxic depending on the initial targets and the extent of activation. Moreno-Navarrete (2019) mentioned that in Alzheimer's disease patients, complement protein synthesis and activation induce neuroinflammation, neuronal and synapse loss, and neurodegeneration. Complement proteins were discovered colocalized with A plaques, which might be the result of an accumulation, which overwhelms the complement system. According to recent study, A1-42 can directly activate the classical pathway by binding to C1q via its globular domain. Mortensen et al. (2017) speculate that complement activation produced by C1q binding to A may contribute to neuroinflammation and neuro-degeneration. Other investigations demonstrated that the complement system is required for synaptic pruning and in a normal brain may contribute to synaptic plasticity throughout lifetime, nevertheless injuries and A buildup might over activate or cause the complement to malfunction later in life (Ma et al., 2013).

5. CONCLUSIONS

Aluminum chloride caused observable oxidative stress and neuro-inflammation, as seen by substantial increases in NO and amyloid beta (Aß) and decreases in SOD and CAT activity in brain tissue.

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