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Alzheimer's Rats Treated Wth Aluminum Chloride Show Damage to the Excretory System: An Evidence-based Approach in Support of Systemic Disruption

Raziyeh Eslami Bashciz[1], Manizheh Karami[1,2*], Haniyeh Amini[1]

1. Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran
2. Neurophysiology Research Center, Shahed University, Tehran, Iran

Abstract

Background and Objective: Many organs such as the excretory system are impaired in Alzheimer's disease (AD), but there are insufficient research and information on these criteria. Researchers use AlCl3 (Al) to induce AD in an experimental model. We investigated the effects of oral Al consumption on the excretory system of rats.

Materials and Methods: The experimental groups received Al (10 and 50 mg/kg) in drinking water for two and four weeks. Each period had a control group that received only water. At the end, blood samples were taken from the hearts of rats by intraperitoneal injection of ketamine and xylazine, and serum was prepared for analysis of levels of uric acid, urea, C-reactive protein, creatinine, albumin, cortisol, IL-1β, and TNF-α. The kidneys were surgically removed from the rats under deep anesthesia and placed in 10% formalin. Kidney tissue was cut and stained with Hematoxylin and Eosin, Evans blue, fast silver nitrate, and the density of corticosteroid receptor in kidney tissue was determined by immunohistochemistry. All data were analyzed using ANOVA.

Results: The results showed that animals treated with Al had a destructive effect on kidney tissue in both periods compared to the control group (significant reduction of capillary network inside Bowman's capsule) and serum levels of cortisol, CRP, IL-1β and uric acid increased, but the levels of albumin, TNF-α and also the expression level of corticosteroid receptors decreased ($P<0.05$).

Conclusion: AD has an adverse effect on kidney structure and function in an animal model, possibly through high levels of IL-1β.

Keywords: Alzheimer's Disease, Aluminum Chloride, Kidney, IL-1β

1. Introduction

Alzheimer's disease (AD) is listed as a global health priority by the World Health Organization (WHO). As the average age of the world population grows, the incidence of AD is increasing, while researchers have not yet discovered the exact cause (1). AD is an age-related neurodegenerative disease characterized by the presence of β amyloid plaques (Aβ) and neurofibrillary tangles (NFT) in brain regions. The first stage of AD is mainly characterized by short-term memory loss, and in more advanced stages, it displays confusion, aggression, mood changes, long-term memory loss, and social withdrawal (2). The histological

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***Corresponding Author:** Manizheh Karami **E-mail:** karami@shahed.ac.ir

characterization of AD is not well described, but the disease has been described as a multifactorial disorder (3). One of the causes of this disease is the accumulation of heavy metals such as aluminum in the brain tissue. Aluminum is one of the most abundant neurotoxic metals on Earth, widely available to humans, and has been repeatedly shown to accumulate in AD-susceptible neuronal foci. Consuming small amounts of this metal during life causes its selective accumulation in brain tissues such as the hippocampus. Also, experimental evidence has shown that chronic exposure to the metal reveals the neuropathological feature of AD (4). If we consider that the cholinergic system, especially in the hippocampus and cerebral cortex, is responsible for memory and learning, the fact that the heavy metals are a potent cholinotoxin, confirms the link between it and AD (2).

Sources of aluminum are corn, yellow cheese, salt, medicinal plants, spices, tea, cosmetics, disposable dishes. It is also present in medicines and is added to drinking water for purification, which easily reaches the body through water. Some medicinal products also contain this metal, and its salts may bind to DNA and RNA, inhibit enzymes such as hexokinase, acid and alkaline phosphatase, phosphodiesterase, and phosphooxidase, and cause heart and kidney and nerve poisoning (2). Excretory system includes kidney, bladder and urethra. Each person normally has two kidneys, which are located on both sides of the vertebral column. Although the kidneys are small, they perform complex and vital functions, maintaining the balance of electrolytes and regulating the volume of fluids in the body. Kidneys receive a high proportion of blood, about 20% of cardiac output, and filter a large volume of blood. Kidney filtering units are mainly located in the renal cortex. The unique renal filtration barrier consists of the glomerular membrane, podocyte cells and the basement membrane of these two types of cells. This fine structure should prevent the filtering of large proteins in the blood such as albumin and immunoglobulins, otherwise, pathological conditions appear. According to the mentioned information and considering the importance of the kidney's role in the body, it is necessary to investigate kidney diseases, including possible inflammations caused by Alzheimer's, so that if needed, an intervention can be done to improve kidney function. In this research, the kidney system of rat model was studied using AlCl3 (Al) consumption in rats.

2. Material & Methods

2.1. Animals

Forty-eight male Wistar aged rats were used in this research (9-10 weeks old, weighing approximately 250 g, purchased from the Pasteur Institute of Iran, Tehran). These animals were placed in standard cages with metal lids. The cages were kept in an animal room at a temperature of 23 ± 2 °C and humidity of 55 % ± 15 %. The lights in the room were turned on from 7:00 to 19:00 to set a 12-hour light-dark cycle. Animals had free access to food and water and were checked daily between 9:00 a.m. and 11:00 a.m. All the standard rules on animal use and care were respected and the ethical code was granted by local Ethics committee at Shaded University (IR.SHAHED.REC.1401.040).

2.2. Animal grouping

After one week of acclimatization, the rats were randomly divided into control and treatment groups. Treatments were done in periods of two and four weeks, and in each of these periods, we had both the control group and the groups receiving doses of 10 and 50 mg/kg of Al (n=8 for each group). All six groups are listed below:

Two-week control group: This group consisted of eight rats, which were placed in groups of two in a total of four cages in the animal room and received normal drinking water for two weeks.

Four-week control group: They received normal drinking water for four weeks. Same as the control group of two weeks treatment, these eight rats were in four cages.

The group receiving Al (10 mg/kg) for two weeks: This group received AlCl3 (10 mg/kg) for two weeks.

The group receiving Al (50 mg/kg) for two weeks: This group received AlCl3 (50 mg/kg) for two weeks.

The group receiving Al (10 mg/kg) for four weeks: This group received AlCl3 (10 mg/kg) for four weeks.

The group receiving Al (50 mg/kg) for four weeks: This group received AlCl3 (50 mg/kg) for four weeks.

All groups were checked daily and a total of 48 male Wistar rats were subjected to this research.

2.3. Materials used

Hydrated aluminum chloride was purchased from Merck, Germany, ketamine and xylazine were obtained from Iran Veterinary Organization with

official permission, Hematoxylin and Eosin were purchased from Arman Co., Iran, Evans Blue and silver nitrate were obtained from Merck, Germany, and specific antibody for corticosteroid receptor (primary, Ab183127) was purchased from Abcam Co., UK.

2.4. Sampling method

2.4.1. Anesthesia and tissue sample preparation and staining

At the end of each treatment period, rats were anesthetized by intraperitoneal injection of ketamine and xylazine. The amount of ketamine and xylazine injected was proportional to the weight of the rats, so their weight was first measured using a special animal scale (with an accuracy of 1 g). Blood samples were taken from the hearts of rats under deep anesthesia. After half an hour, when the blood clotted, the blood was centrifuged at 3000 rpm for about 15 minutes, then the serum was separated and placed in a freezer at -80ºC, and within a maximum of two weeks, relevant tests were completed. The serum levels of uric acid, urea, C reactive protein (CRP), cortisol, IL-1β, and TNF-α were measured by ELISA or relevant kit. Following the collection of blood, the animal underwent surgery (kidney separation) and the tissue samples of the control and treatment groups were placed in 10% formalin.

2.4.2. Tissue sample preparation

After fixation (about 72 hours), tissue processing followed and sections (3-4 µ) were prepared. Some slices were mounted on slides charged with poly-L-lysine. The samples were stained after 24 hours in the following steps: Deparaffinization with xylene (twice, 5 minutes each), hydration in alcohols 96%, 80%, 70%, and 50% for 5 minutes each. After this step, the samples were ready for staining.

2.4.3. Hematoxylin-Eosin (H&E) staining

After deparaffinization and hydration with decreasing degrees of alcohol, the sections were placed in 20% hematoxylin stain for 30 minutes, then washed with distilled water, placed in eosin for 20 minutes, and rinsed in distilled water. The slices were dehydrated in alcohols with ascending degrees (*i.e.* in alcohols of 50%, 70%, 80% and 90%, 2 minutes each). At the end, they were cleared twice in xylene, mounted with Entellan (Merck, Germany) and covered with coverslips.

2.4.4. Evans blue staining

The stain was first prepared by dissolving 0.25 g of Evans blue powder in 100 mL of $CaCl_2$ buffer (0.1 M, pH = 5.6). The solution was shaken, filtered using filter paper, and poured into a dark container. To begin staining, after deparaffinizing the tissue with xylene for 30 minutes and hydration with reducing alcohols for 5 minutes each, using a pipette, a few drops of Evans blue staining solution poured onto the sections and placed in a dark humidified incubator (37°C) for 1.5 hours. Then, after washing with distilled water, dehydrating with ascending grades of alcohol for 1-2 minutes and clarifying with xylene for two minutes, the slides were mounted with glue (Entellan, Merck).

2.4.5. Silver nitrate staining (fast kit)

First, the dye was dissolved in distilled water, filtered using filter paper, and placed in a dark container. When used, it was first sonicated for 30 minutes and immediately placed on the slices for 30 minutes. Then, the slides were washed in distilled water for five seconds, and incubated at 37ºC. After 30 minutes, the slides were cleaned again in distilled water and exposed to the light of a desk lamp (100 W) or sunlight to find the desired color. Then, they were placed in increasing alcohols for two minutes each, and in the next step, they were clarified with xylenes (3 minutes each), and finally mounted and covered.

2.4.6. Corticosteroid receptor immunohistochemical staining

The samples mounted on poly-L-lysine-charged slides were deparaffinized, hydrated, and placed in phosphate-buffered saline (PBS: pH=6.7) for 1–2 minutes and then temporarily immersed in 0.03% Triton X-100 solution. After that, the slides were exposed to specific antibody (Ab183127, Abcam) for one hour in Bain-Marie (37ºC). The slides were then washed four times in buffer and avidin was added to the samples to enhance the target antigen and placed for 10 minutes in laboratory conditions. The slides were then exposed to counterstain for 10 minutes, washed with buffer and then with distilled water and alcohol. They were finally cleared in xylene for two minutes and mounted.

2.4.7. Cortisol concentration measurement by ELISA method

All reagents, working standards and samples were prepared according to the kit instructions

and placed at room temperature for 20 minutes. In this assay, 50 µL of sample and standard were added to each well, and immediately 50 µL of primary antibody was added to all wells and shaken for 30 seconds and then incubated for 40 minutes at 37°C. The plate was emptied and washed four times each time with 300 µL of wash solution in the plate kit. 50 µL of Horseradish peroxidase (HRP)-conjugated secondary antibody solution was added to each well and incubated for 30 minutes at 37°C. The plate was emptied and washed four times each time with 300 µL of wash solution in the plate kit. 50 µL of HRP-conjugated streptavidin solution was added to each well and incubated for 30 minutes at 20-25°C. The plate was emptied and washed four times each time with 300 µL of wash solution in the plate kit. Then, 90 µL of substrate solution was added to each well and incubated for 20 minutes in the dark at 37°C, thereafter, 50 µL of test stop solution was added to each well. Finally, using an ELISA device, optical absorbance was read at a wavelength of 450 nm, and the data based on the standard curve was used to plot cortisol concentration.

2.4.8. Measurement of urea, creatinine, albumin, uric acid and C-reactive protein (CRP)

Blood chemistry was calculated with an autoanalyzer. The necessary tests for the autoanalyzer were defined and factor levels were measured.

2.4.9. TNF-alpha assay with ELISA

All reagents and working standards and samples were prepared according to the kit instructions and placed at room temperature for 20 minutes. Then, 100 µL of sample and standard were added to each well and incubated for 2 hours at room temperature. The plate was emptied and washed three times with 400 µL of wash solution. 100 µL of TNF-α conjugated solution was added to each well and incubated for 2 hours at room temperature. The plate was emptied and washed three times each time with 400 µL of wash solution. 100 µL of Streptavidin-HRP B solution was added to each well and incubated for 20 minutes at room temperature. The plate was emptied and washed three times each time with 400 µL of plate wash solution. 100 µL of substrate solution was added to each well and incubated for 20 minutes in the dark. 50 µL of stop solution was added to each well. Finally, an ELISA device was used to read the absorbance at a wavelength of 450 nm, the data was entered into Excel, a standard curve was drawn based on the optical absorbance, and the TNF-α concentration was measured.

2.4.10. IL-1β assay by ELISA

All the reagents, working standards, and samples were prepared according to the kit instructions and placed at room temperature for 20 minutes. 50 µL of the dilution solution was added to all wells and 50 µL of the sample and standard were added to each well and incubated for 2 hours at room temperature. The plate was emptied and washed three times, each time with 400 µL of plate wash solution. 100 µL of IL-1β conjugated solution was added to each well and incubated for 2 hours at room temperature. The plate was emptied and washed three times, each time with 400 µL of the wash solution. 100 µL of substrate solution was added to each well and incubated in the dark for 20 minutes. 100 µL of stop solution was added to each well. Using an ELISA device, the absorbance was read at a wavelength of 450 nm and the data was entered into Excel. Based on the absorbance, a standard curve was drawn and finally the concentration of IL-1β in each well was measured.

2.5. Statistical analysis of data

Data were analyzed using SPSS software (version 22). After Kolmogorov-Smirnov test, findings were calculated with analysis of variance (ANOVA). Tukey's *post hoc* test was used to compare the groups with the control group. $P<0.05$ was considered as significant value. Images were evaluated by ImageJ Java software.

3. Results

The effect of oral consumption of AlCl3 (Al) with different doses (10 and 50 mg/kg) at different time intervals (2 weeks and 4 weeks) on the serum levels of cortisol, albumin, creatinine, urea, uric acid, CRP, TNFα, Il-1β has been reported as follows:

3.1. Serum cortisol level

No significant difference in cortisol concentration was observed in the groups treated with Al for two weeks, but under a dose of 50 mg/kg during four weeks of treatment, a significant increase was shown compared to the control group (Figure 1).

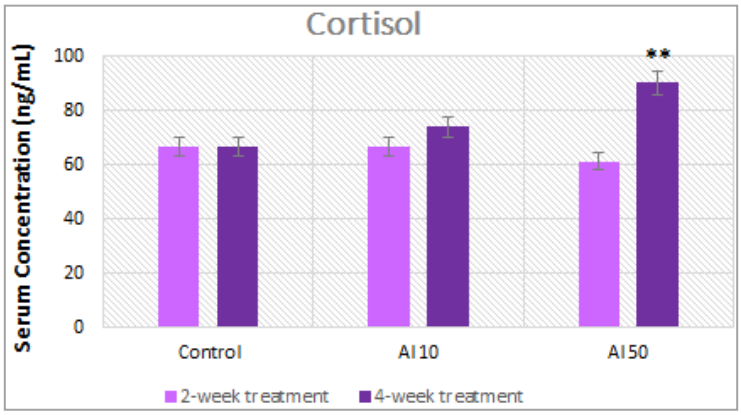


Fig. 1. Serum concentration of cortisol. The control group only received normal drinking water. Experimental groups received Al (10 and 50 mg/kg) orally for two and four weeks, Asterisks were obtained based on Tukey's *post hoc* test (**P<0.01 *vs.* control).

3.2. Urea and C-reactive protein serum levels in treatment and control groups

The results of serum concentration evaluation among different groups showed that in low dose groups, urea concentration did not change significantly regardless of the consumption period, but a relative decrease was observed in high dose treatment groups. C-reactive protein (CRP) had a relative increase compared to the control (without the effect of dose and duration of use) (Figure 2).

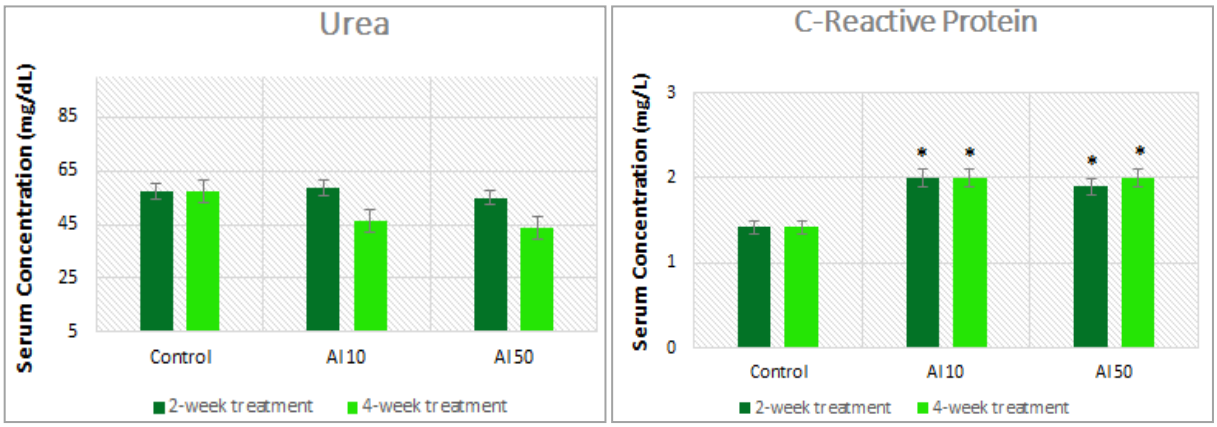


Fig. 2. Serum concentration of urea and CRP. The control group only received normal drinking water. The other groups received Al (10 and 50 mg/kg) orally for two and four weeks. Urea showed a relative decrease in high doses (without effect of duration of use). CRP showed a relative increase compared to the control (*P<0.05).

3.3. Serum levels of creatinine, albumin and uric acid in treatment and control groups

The results of serum concentration evaluation among different groups showed that there was a relative increase in uric acid concentration in Al treatment groups (Figure 3). Also, the results showed no significant change in albumin concentration in the low dose group (10 mg/kg) in the 2-week period. However, in the 50 mg/kg aluminum group, a relative decrease in the albumin concentration was observed over the 4-week period (Figure 3).

No significant change in creatinine concentration was observed in all treatment groups compared to the control regardless of the dosage and duration of use (Figure 3).

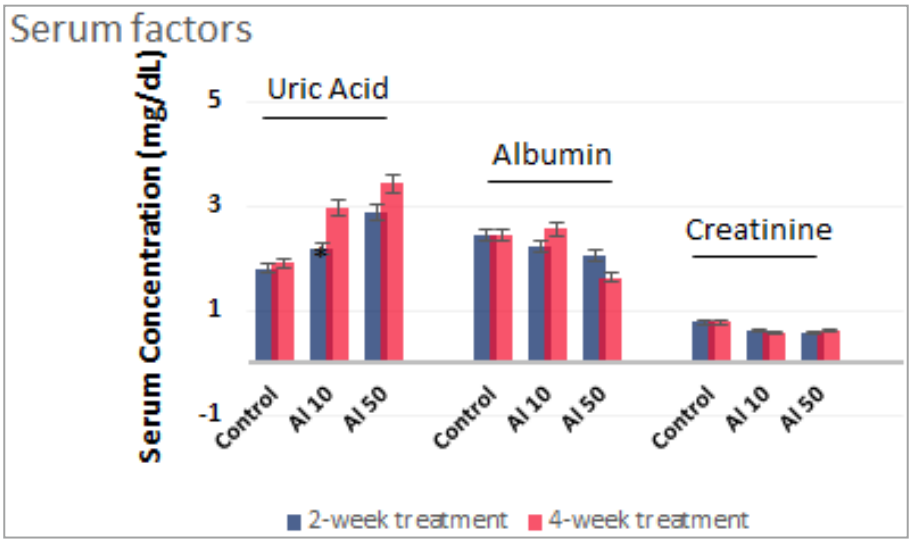


Fig. 3. Serum albumin, uric acid and creatinine. The control group only received normal drinking water. Others were treated by Al (10 and 50 mg/kg) orally, one group for two weeks and the other group for four weeks. A relative increase in uric acid but a relative decrease in albumin (high Al dose) was observed.

3.4. TNF-alpha serum level in treatment and control groups

A significant decrease in the concentration of TNF-α was observed compared with the control group in the groups treated with Al, regardless of the dose and duration of Al consumption (Figure 4).

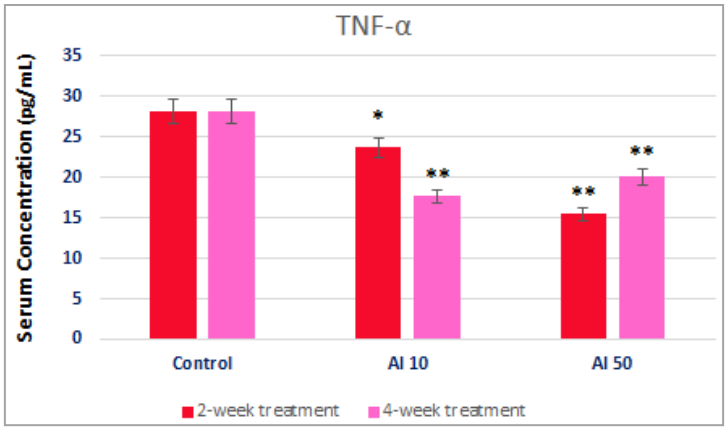


Fig. 4. Serum concentration of TNF-α. The control group only received normal drinking water, other groups received Al (10 and 50 mg/kg), orally for two and four weeks. Asterisks were obtained based on Tukey's *post hoc* test (*P<0.05, **P<0.01 *vs*. control).

3.5. IL-1β serum level in treatment and control groups

The results of the evaluation of serum concentration among different groups showed that a significant increase was observed in the two-week and 4-week Al treatment groups compared to the control (Figure 5).

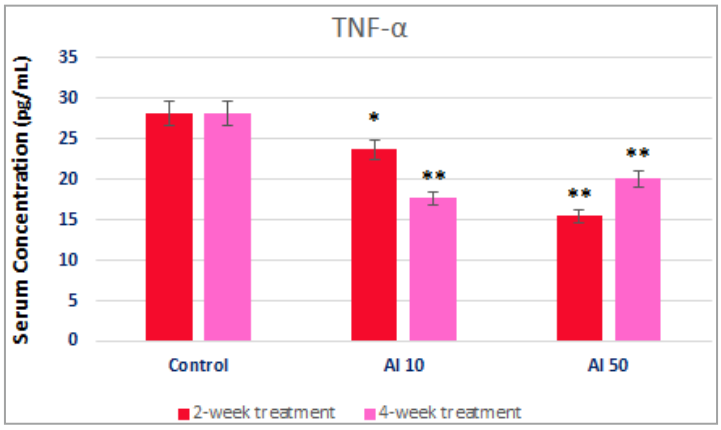


Fig. 5. IL-1β serum concentration. The control group only received normal drinking water. Other groups consumed Al (10 and 50 mg/kg) orally, for two and four weeks. Asterisks were obtained based on Tukey's *post hoc* test. (*P<0.05, **P<0.01 *vs.* control).

3.6. Glomerular tufts to Bowman's capsule (G/B)

The effects of oral Al intake at different doses (10 and 50 mg/kg) and different time intervals on the ratio of glomerular tufts to Bowman's capsule were investigated using ImageJ Java software using microscopic sections of the kidney at 400x magnification (Figure 6). Also, a number of 200 µm tissue samples were randomly analyzed. Bowman's capsule without glomerular tufts, capsular lesions, interstitial tubule lesions, and vascular lesions are shown in Figure 6.

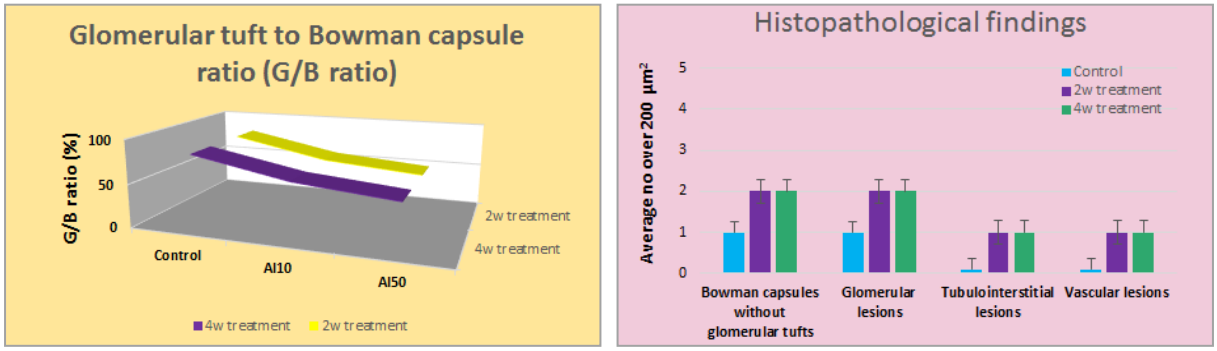


Fig. 6. The ratio of glomerular tufts to Bowman's capsule in terms of percentage and other structural characteristics of the kidney. The control group only received normal drinking water. The treatment groups received Al (10 and 50 mg/kg) orally, for two and four weeks. G/B showed a decrease in the two-week and four-week treatment groups compared to the control. Other histopathological results are also shown in the graph.

3.7. Tissue investigations with general hematoxylin-eosin (H&E) staining

Figure 7 shows a cross-section of the kidney tissue in a rat treated with Al under H&E staining. The treated animal (2 weeks) had vascular destruction.

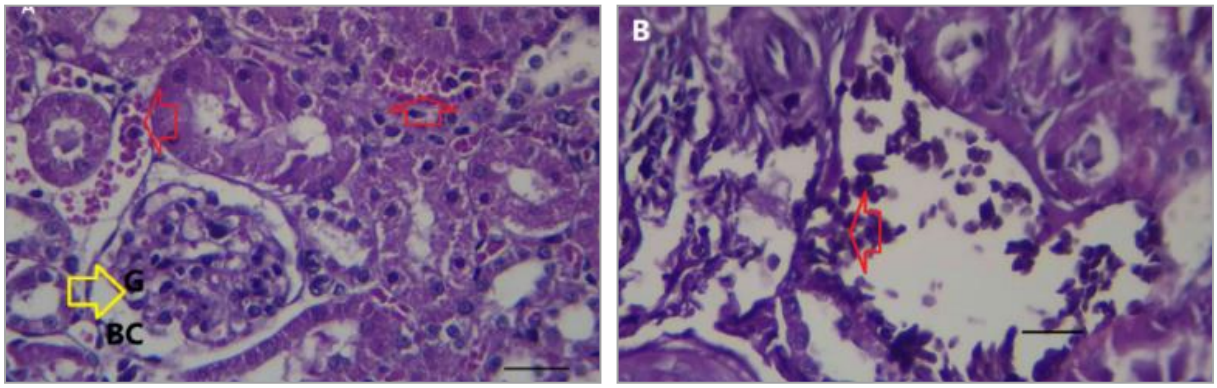


Fig. 7. H&E staining of the cross section of kidney tissue. Control group without tissue damage (A). An animal treated for two weeks showed vascular injury (B) (G: glomerulus, BC: Bowman's capsule, note red arrows). Scale bar: 50 µm.

3.8. Histological investigations with Evans blue staining

Evans blue solution binds to albumin inside the samples and then turns into a high molecular weight protein tracer. We used this staining to show albumin content in the kidney. A transverse section of kidney tissue (Figure 8) showed a relative increase in renal albumin content in rats treated with Al.

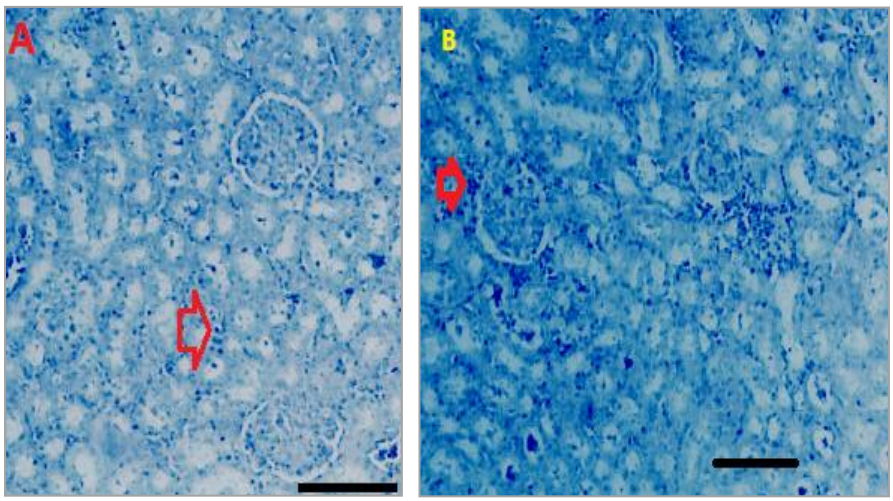


Fig. 8. Transverse section of rat kidney tissue stained with Evans blue. The control sample (A) has no tissue damage and for the treatment group (2 weeks) (B) has a relative increase in kidney albumin content. Scale bar: 100 µm.

3.9. Histological examination with fast silver nitrate staining

Figure 9 shows the cross section of kidney tissue in rats. This stain is used to clarify Bowman's space. The capsule space increased in the Al-treated groups.

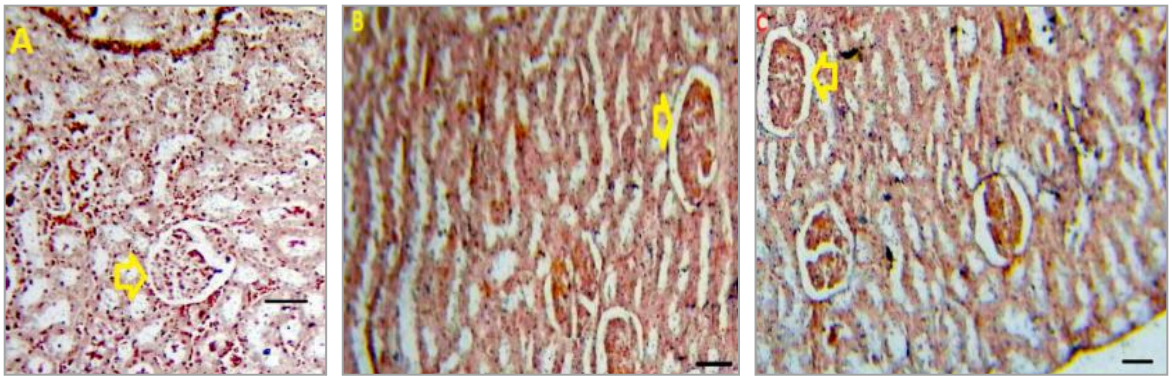
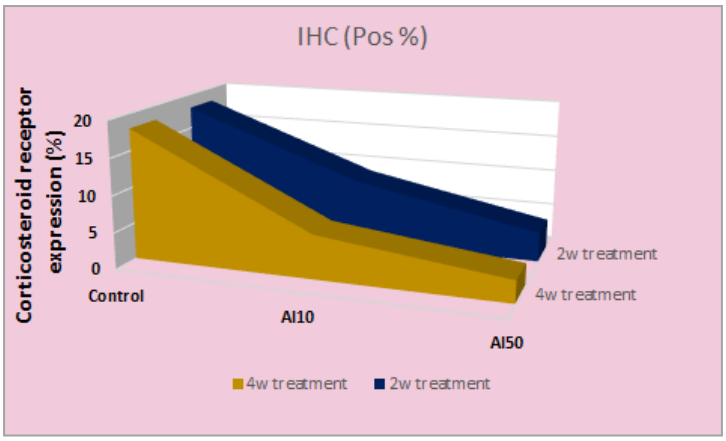


Fig. 9. Section of kidney tissue in rat. No tissue damage was observed in the control group (A), but a relative increase in space was observed with Al higher doses (50 mg/kg) for two (B) and for four weeks (C). Scale bar: 100 µm.

3.10. Histological examination of corticosteroid receptor by immunohistochemical staining method

The glucocorticoid receptor (GR) belongs to nuclear steroid receptors and acts as a ligand-dependent transcription factor. The concentration of GR expression in the kidney tissue section was studied by immunohistochemical staining and is shown in Figure 10. A decrease in the expression ratio of that protein in Al-treated animals was shown compared to the control and was correlated with the dose and duration of treatment.



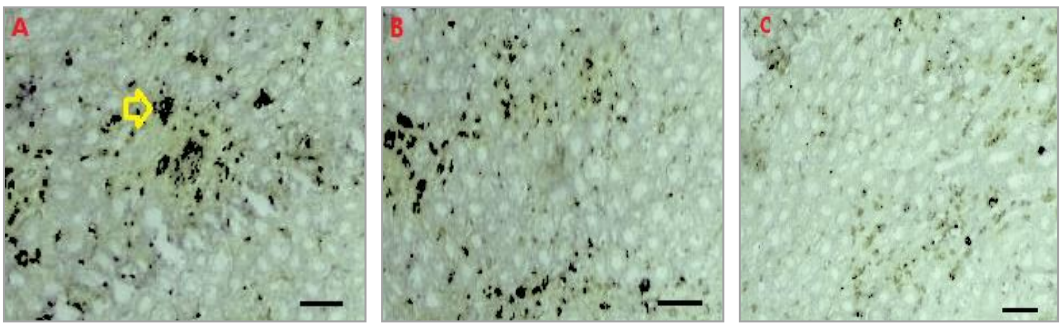


Fig. 10. Glucocorticosteroid receptor (GR) protein expression ratio by immunohistochemical staining. A decrease in the expression ratio of GR protein was shown in Al-treated animals compared to control animals and was correlated with the dose and duration of treatment. Micrographs are for control (A) and Al (50 mg/kg) for two and four weeks (B and C). (The reduction is more evident at 4 weeks). Scale bar: 50 µm.

4. Discussion

The aim of this study was to investigate the damage of the excretory system (kidney) in Alzheimer's rats treated with aluminum chloride (Al) and the results of this study showed that the animal treated with Al had damage in the kidney tissue compared to the control group (*e.g.* significant reduction of the capillary network inside Bowman's capsule). Also, the serum levels of cortisol, CRP, IL-1β, and uric acid increased, and the levels of urea, creatinine (not significantly), albumin, TNF-α, as well as the glucocorticosteroid receptor (GR) protein decreased. Based on the present data, the weight of rats did not show significant changes (not shown).

It has been reported that Al enters the body through two main routes, pulmonary and oral. Although only a small part of Al is absorbed through the gastrointestinal tract, oral consumption is associated with the greatest toxicological consequences. There are approximately one million nephrons in the human kidney. Their collective function is to maintain the homeostasis of ions and small molecules in the blood. The first part of the nephron is the glomerulus, a capillary "tuft" enclosed in Bowman's capsule. The capillary network performs the primary filtration of water, ions, and small molecules from the blood. In a healthy person, 20% of the cardiac output goes to the kidneys, of which 10-15% is filtered by the glomeruli (glomerular filtration rate). The filtrate passes through the tubular system of the nephron, where needed substances are absorbed and ion concentrations are regulated, and toxic and unnecessary substances are excreted. Glomeruli are damaged by autoimmune diseases or toxic chemicals and also due to diseases such as diabetes and hypertension (5).

Based on the results of this research, tissue damage was revealed in the kidney tissue and the ratio of glomerular tufts to Bowman's capsule (G/B) decreased and also the average of Bowman's capsule without glomerular tufts increased. In addition, both glomerular and interstitial tubules and vascular damage were observed, indicating the toxic effect of Al in the kidneys in the AD model. According to previous studies, one of the main target organs of Al is the kidneys (6). Our results on kidney tissue damage are somewhat consistent with that study, but the functional damage shown in ours is interesting: The levels of cortisol, uric acid, CRP and an important inflammatory factor, IL-1β, were increased in Al-treated rats. Some factors such as urea, albumin, creatinine and TNF-α showed a decrease (the first three factors had a relative decrease, but TNF-α decreased significantly).

The increase in cortisol level is consistent with previous research (7). Cortisol is secreted from the adrenal cortex. Glucocorticoids represent a family of endogenous pleiotropic steroid hormones that are essential for life and regulate a wide range of vital physiological processes, *e.g.* the response to a diverse set of stressors. They act by binding to the glucocorticoid receptors (GRs) and mediates a wide range of cellular functions. Cortisol homeostasis is itself dynamic and involves multiple mechanisms, primarily the hypothalamic-pituitary-adrenal (HPA) axis (7). Previous studies have shown that the ratio of circulating cortisol to corticosterone may be affected by physiological conditions, developmental stages, and organ differences. It has been shown that cortisol is faster than corticosterone during severe acute stress and corticosterones are involved in chronic stress (8). With this description, we had to calculate these ratios, so it is considered one of the limitations of our research.

According to the mentioned findings and according to the specific immunohistochemical staining results in this research that show a decrease in GR protein expression in rats treated with Al, it can be concluded that the increase in exposure to Al (2 weeks and 4 weeks) decreased cortisol receptors and cortisol-receptor interaction. But there may be receptor resistance, although, this seems probable.

Albumin is a simple protein (without carbohydrates) consisting of a polypeptide chain with 585 amino acids. Daily about nine to 12 g of albumin is synthesized by the liver and enters the blood circulation. Albumin is the most abundant protein in the blood circulation. Due to its relatively small size and high plasma concentration, albumin is an important component of most extravascular fluids such as cerebrospinal fluid (CSF), interstitial fluid, urine and amniotic fluid during pregnancy. It plays an essential role in maintaining plasma oncotic pressure, binding to various ligands, providing amino acids needed by tissues. In cases such as malabsorption and malnutrition, liver diseases, abnormal excretion of albumin due to kidney disease and intestinal inflammation, the blood albumin level decreases. Albumin measurement is very important in the assessment of malnutrition, monitoring of hyperglycemia treatment, detection and monitoring of kidney diseases (9). In the present study, serum albumin levels showed a

decrease in high Al dose (50 mg/kg) over a 4-week period, which is consistent with previous studies (8). Also, other indicators of kidney damage such as serum levels of uric acid, urea and creatinine were also investigated, where uric acid increased relatively, but creatinine and urea decreased relatively. In a previous study, it has been shown that in rats receiving AlCl3 (for 40 days by gavage), serum levels of urea and creatinine increased and creatinine clearance decreased (10). As this study has shown, this substance is mainly excreted through the kidneys, causing marked tubular degeneration. An increase in the concentration of serum urea and creatinine can be the result of the critical accumulation of this metal in the kidneys and eventually lead to kidney failure (10). It has also been shown in previous studies that treatment with Al causes a significant increase in plasma glucose and creatinine levels, while the urea level decreases significantly compared to the control (11). Therefore, there are different results and some authors have reported urea reduction similar to our findings, and maybe this part of the results should be repeated, which is not possible at the moment. C-reactive protein (CRP), a pentameric protein composed of five identical subunits, is the primary protein of the acute phase in response to infection and inflammation. CRP is synthesized in the liver and serves as a reliable biochemical marker for systemic inflammation in clinical practice. Recent studies also show that CRP can be produced by many inflammatory cells, such as inflammatory macrophages. In renal diseases, CRP is highly expressed by many inflammatory cells, possibly macrophages, and renal intrinsic cells including tubular cells and endothelial cells. In acute infection or inflammation, CRP can be secreted at the beginning of four to 10 hours after inflammatory stimulation and reaches its peak in 48 hours and has a short half-life of 19 hours (12). Considering high CRP and low serum albumin, the ratio of CRP to albumin is considered as an important prognostic indicator in critically ill patients (12, 13). The current results show an increase in CRP and a decrease in albumin, consistent with those studies, which may reflect the onset of renal inflammation.

Inflammation is caused by a group of secreted polypeptides called cytokines. In the kidney, cytokines induce the expression of reactive oxygen/nitrogen species, bioactive lipids, and adhesion molecules (14). Cytokines are proteins secreted by innate and adaptive immune cells that mediate many of these cells' actions. These molecules are produced in response to antigens and other microorganisms. In the present study, the serum level of pro-inflammatory cytokine IL-1β was increased. IL-1β is a proinflammatory cytokine produced by macrophages, monocytes, and epithelial cells in response to infections, microbial invasion, and inflammation to aid immunity (15). The significant increase of this inflammatory factor confirms the assumption (start of inflammatory response) and therefore the high level of this molecule can indicate inflammation. Tumor Necrosis Factor-Alfa (TNF-α) is one of the main inflammatory cytokines and plays a central role in host defense, inflammation and immune system function, which is associated with pathogenesis, development and progression of various infections, diseases. This molecule is produced by macrophages, monocytes, neutrophils, T cells and NK cells (16), and is related to autoimmunity, and malignant diseases (17). Studies conducted in the field of kidney inflammation and nervous system inflammation have reported an increase in TNF-α (17), which is not consistent with our results. This difference may be due to experimental design, animal model, dose and method of administration of Al, or even error in the analysis, which needs to be repeated. But it should not be ignored that its high level clearly indicates pathological complications such as autoimmune diseases (18). Recently, the role of TNF-α and its increased levels in biological fluids in cognitive disorders has been discussed, and it has been suggested that treatment with TNF-α blocking agents is more effective in eliminating systemic inflammation and may be effective in preventing AD (19). So, any definitive opinion about it and AD depends on further studies.

Conclusion

Oral administration of AlCl3 (Al) with water at doses of 10 and 50 mg/kg for two and four weeks to induce Alzheimer's causes damage to the capillary network of Bowman's capsule and renal vessels. It increases the serum level of cortisol, CRP, uric acid and pro-inflammatory factor Il-1β, which has destructive effects on kidney function.

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References

[1] Sinyor B, Mineo J, Ochner C. Alzheimer's disease, inflammation, and the role of antioxidants. The Journal of Alzheimer's Disease Reports. 2020; 4: 175-83.

[2] Mathiyazahan DB, Thenmozhi AJ. Protective effect of black tea extract against aluminium chloride-induced Alzheimer's disease in rats: A behavioral, biochemical and molecular approach. Journal of Functional Foods. 2015; 16: 423-435.

[3] Liu Y, Meng X, Sun L, Pei K, Chen L, Zhang S, Hu M. Protective effects of hydroxy-α-sanshool from the pericarp of *Zanthoxylum bungeanum* Maxim. On D-galactose/AlCl3-induced Alzheimer's disease-like mice via Nrf2/HO-1 signaling pathways. European Journal of Pharmacology. 2022; 5: 914.

[4] Tomljenovic L. Aluminum and Alzheimer's disease: after a century of controversy, is there a plausible link? Journal of Alzheimer's Disease. 2011; 23: 567-98.

[5] Terasaki M, Brunson JC, Sardi J. Analysis of the three dimensional structure of the kidney glomerulus capillary network. Scientic Reports. 2020; 10: 20334.

[6] Al Dera HS. Protective effect of resveratrol against aluminum chloride induced nephrotoxicity in rats. Saudi Medical Journal. 2016; 37: 369-78.

[7] Sagmeister MS, Harper L, Hardy RS. Cortisol excess in chronic kidney disease - A review of changes and impact on mortality. Frontiers in Endocrinology (Lausanne). 2023; 13: e1075809.

[8] Gong S, Miao YL, Jiao GZ, Sun MJ, Li H, Lin J, Luo MJ, Tan JH. Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. PLoS One. 2015; 10: e0117503.

[9] Hussein MM, Ali HA. Aluminum–induced oxidative stress and hepato-renal impairment in male albino rats: possible protective trial with naringenin. Life Sciences. 2016; 13: 93-104.

[10] Balgoon MJ. Assessment of the protective effect of *Lepidium sativum* against aluminum-induced liver and kidney effects in albino rat. BioMed Research International. 2019: e4516730, doi.org/10.1155/2019/4516730.

[11] Belaïd-Nouira Y, Bakhta H, Haouas Z, Flehi-Slim I, Ben Cheikh H. Fenugreek seeds reduce aluminum toxicity associated with renal failure in rats. Nutrition Research and Practice. 2013; 7: 466-74.

[12] Li J, Chen J, Lan HY, Tang Y. Role of C-Reactive Protein in Kidney Diseases. Kidney Diseases (Basel). 2022; 9: 73-81.

[13] Yu J, Park JY, Ha S, Hwang JH, Kim YK. C-reactive protein/albumin ratio and acute kidney injury after radical cystectomy among elderly patients: A propensity score-matched analysis. Disease Markers. 2020: e8818445.

[14] Amdur RL, Feldman HI, Gupta J, Yang W, Kanetsky P, Shlipak M, *et al.* Inflammation and progression of CKD: the CRIC study. Clinical Journal of the American Society of Nephrology. 2016; 11: 1546–1556.

[15] Tartibian B, Kamrani A, Yaghoobnezhad F, Amini Khayat SM. Investigating the relationship between sleep quality and interleukin-1 beta and C-reactive protein in active and inactive men. Jouernal of Rafsanjan University of Medical Sciences. 2014; 13: 141-150.

[16] Panigrahi R, Sarkar N, Biswas A, Pal A, Saha D, Singh SP, *et al.* Association of TNF-α promoter polymorphism with HBV associated disease outcome among HBV infected patients from Orissa, Southern part of East India. Journal of Clinical and Experimental Hepatology. 2014; 4: 202-208.

[17] Sahraean Z. Expression pattern of tumor necrosis factor-α (TNF-α) under the influence of *Zingiber officinale* hydroalcoholic extract. Journal of Animal Physiology and Devlopement (Biological Sciences). 2017; 11: 41-53.

[18] Jang D, Lee A-H, Shin H-Y, Song H-R, Park J-H, Kang T-b, *et al.* The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. International Journal of Molecular Sciences. 2021; 22: 2719.

[19] Plantone D, Pardini M, Righi D, Manco C, Colombo BM, De Stefano N. The role of TNF-α in Alzheimer's disease: a narrative review. Cells. 2024; 13: 54. doi.org/10.3390/cells13010054.