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Non-linear histological alteration in the duodenum, Hepar, and renal mice following oral Aluminium exposure at varying doses

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Abstract

This study aims to investigate the effects of Al exposure on the duodenum, liver, renal function, and survival rate. Aluminium (Al) is one of the heavy metals that is widely used in daily life and has a negative impact on health. A total of 51 male Balb/c mice, aged 3 months, were randomly assigned to four groups. The negative control group was administered sterile aquadest, while the treatment groups received oral administration of AlCl3 at doses of 100 mg/kg body weight (BW), 150 mg/kg BW, and 200 mg/kg BW, respectively, for 53 consecutive days via oral gavage. The ANOVA and Kruskal-Wallis tests were used in statistical analyses. The duodenal inflammation score, the severity of focal inflammation and nuclear degeneration in the liver, and the glomerular-to-renal corpuscle ratio tended to increase in the 100 mg/kg BW group compared to the control and other treatment groups. Interestingly, the highest mortality rate was not observed in the 100 mg/kg BW group. Aluminium exposure affected the histological features of the duodenum, liver, and renal function, with the most pronounced changes observed in the 100 mg/kg BW group. These findings suggest that the toxic effects of aluminium may not exhibit a linear relationship with increasing doses. Aluminium contamination is a concern even at low doses.

Keywords: Aluminum toxicity, Duodenum, Hepatic, Renal, Public health.

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1. Introduction

Aluminium (Al) is a widely utilized heavy metal that plays a significant role in daily human activities, leading to extensive exposure. Its presence is pervasive due to its natural abundance and widespread use in various industries. Humans are exposed to aluminium through multiple pathways, including polluted air, water, soil, and food. Aluminium compounds are commonly employed in pharmaceuticals, water treatment processes, kitchenware, containers, food additives, consumer goods, appliances, food packaging, and cookware. Primary dietary sources of aluminium include salt, corn, spices, yellow cheese, tea, and herbs. The extensive use of aluminium-containing compounds and their contribution to environmental pollution have led to elevated levels of aluminium exposure in humans, exceeding natural background levels [1, 2]. Al is particularly challenging to eliminate from the environment due to its long-lasting nature, making it difficult for people to protect themselves from metal exposure. This persistent exposure negatively impacts health [3].

Al enters the body through inhalation, ingestion, and parenteral treatment, accumulating in various tissues and exerting toxic effects [3]. However, due to its ubiquitous use in daily life, the acceptable weekly dosage of Al among humans is established at 1 mg Al/kg BW; humans may exceed these health-based guidance values. Although Al absorption through the gastrointestinal tract is low, the cumulative exposure from various sources results in continuous and partly underestimated Al intake. The limited interaction of aluminum with normal biomolecules further renders the body more susceptible to aluminum toxicity [4].

Al toxicity influences multiple organ systems, including the respiratory, cardiovascular, gastrointestinal, haematological, neurological, musculoskeletal, and reproductive systems [3]. In the liver, Al toxicity causes congested central veins with mild fibroplasia and infiltration of inflammatory cells. Distorted sinusoids, periportal edema, and mild fibroplasia are also observed, with inflammatory cells predominantly consisting of lymphocytes. In addition, moderate proliferation of connective tissue (fibroplasia) is observed in conjunction with adenomatous and papillary hyperplasia of bile ductules, as has been reported. Aluminium exposure also leads to alterations in liver biochemical parameters [5-9]. Al toxicity results in tubular dilatation, decreased Bowman's space, congestion, interstitial fibrosis, tubular atrophy, and inflammation in the kidneys. Renal function tests also indicate a decline in kidney function [9-12].

The reproductive system is also affected by aluminum toxicity, leading to reductions in semen parameters, depletion of spermatogenic cells, damage to seminiferous tubule structures, decreased reproductive hormone levels, and increased oxidative stress [1, 2, 13, 14]. Aluminum causes congestion of blood vessels, interfibrillar edema, hemorrhage, and myocardial degeneration in the heart [6].

Studies investigating the effects of Al toxicity have reported varying outcomes depending on the dose, due to the suggested biphasic effects of Al and its unstable impact at human dietary and high exposure levels [4].

In this study, we examine the effects of Al consumption at three distinct doses on three organs – the duodenum, liver, and kidneys – as well as the survival rate.

2. Materials and Methods

2.1. Ethical Approval

The experimental procedure was conducted in accordance with ethical guidelines for using animals in research. Ethical clearance was received from the Research Ethics Committee, Faculty of Medicine, Universitas Airlangga (No. 187/EC/KEPK/FKUA/2023).

2.2. Experimental Design

A total of 51 mice were randomly divided into four groups: one control group and three treatment groups. The control group was administered sterile aquadest by oral gavage for 53 consecutive days. The treatment groups received oral administration of AlCl3 at doses of 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW, respectively, for 53 consecutive days. In the morning, the mice were euthanized under diethyl ether anesthesia. At the end of the study, the duodenum, liver, and kidneys were excised for histological analysis after dissection.

Aluminium chloride ($AlCl_3$) was purchased from Sigma-Aldrich, Germany. AlC_3 powder was dissolved with sterile water for injection (Ikapharmindo Putramas, Jakarta, Indonesia). All solutions were prepared immediately before use.

2.3. Experimental Animals

Male Balb/c mice, 3 months old, were purchased from Pusvetma, a licensed laboratory animal supplier. The mice were housed in laboratory cages at the experimental animal facility of Universitas Airlangga under standard laboratory conditions, with a temperature range of 19°C to 22°C, humidity between 40% and 65%, and a 12-hour light/dark cycle. Throughout the experiment, the mice were provided unlimited access to food and water.

2.4. Histological examination

The duodenal, hepar, and renal tissues were fixed for 24 hours in 4% neutral buffered formalin, then processed and embedded in paraffin wax. Using a rotary microtome (Leica 2125, Chicago, IL, USA), tissues were sectioned at a thickness of 5 µm. An Olympus BX-41 microscope was used for histological examination at 400x magnification. The figures were analyzed with ImageJ. Duodenum histological alteration was assessed for inflammatory cell infiltration and ulceration, as described previously [15]. Hepar histological examination focused on inflammatory cell infiltration and hepatocyte degeneration, as described previously [7]. Renal histology was evaluated for glomerular area and renal corpuscle area, as described previously [16].

2.5. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted using GraphPad Prism (v8.4.3, GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) was performed for parametric data, while non-parametric data were analyzed using the Kruskal-Wallis test, followed by multiple comparisons. A p-value < 0.05 was considered statistically significant.

3. Result

The inflammation score in the duodenum was not significantly different among the groups. The 100 mg/Kg BW group had the highest inflammation score, showing a tendency toward a difference compared to the other groups (Table 1 and Figure 1a). The control group and the other two treatment groups exhibited similar scores.

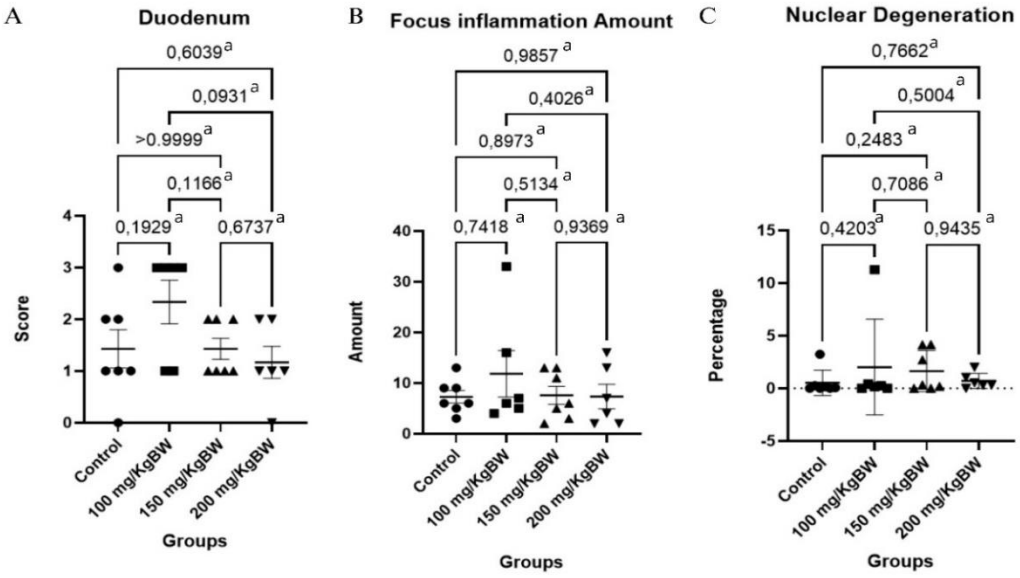

Figure 1.
Statistical analysis of the duodenum and hepar: Duodenum inflammation score (1A), focus inflammation amount of hepar (1B), and hepatocyte nuclear degeneration (1C) after 53 days of treatment. The p-value between the two groups is presented above the bar. Different lowercase letters represent significant differences ($p < 0.05$). (n = 7, control; n = 6, 100 mg/Kg BW; n = 7, 150 mg/Kg BW; n = 6, 200 mg/Kg BW).

Table 1.
Duodenum inflammation score

Groups	Inflammation score (Score ± SEM)
Control	1.43 ± 0.37^a
100 mg/KgBW	2.33 ± 0.42^a
150 mg/KgBW	1.43 ± 0.42^a
200 mg/KgBW	1.17 ± 0.31^a

Source: Results were presented as mean ± SEM for control (n =7), dosage 100 mg/KgBW (n = 6), 150 mg/KgBW (n = 7), and 200 mg/KgBW (n = 6). Statistical analysis was performed using the Kruskal-Wallis test. Different lowercase letters represent significant ($p<0.05$).

The duodenum inflammation score, as shown in Figure 1A, indicates that AlCl3 administration influences the duodenum at all doses, with the most severe impact observed in the 100 mg/Kg BW group. The effect of Al on the liver is presented in Table 2; Figures 1 B and 1 C. Focused inflammation amount and nuclear degeneration were increased in the 100 mg/Kg BW group.

Table 2.
Hepatocyte inflammation and nuclear degenerative.

Groups	Focus Inflammation Amount (∑ ± SEM)	Degenerative Nuclear Percentage (%± SEM)
Control	7.29 ± 1.25^a	0.53 ± 0.45^a
100 mg/KgBW	11.83 ± 4.59^a	2.01 ± 1.85^a
150 mg/KgBW	7.57 ± 1.77^a	1.65 ± 0.74^a
200 mg/KgBW	7.33 ± 2.42^a	0.70 ± 0.30^a

Source: Results were presented as mean ± SEM for control (n =7), dosage 100 mg/KgBW (n = 6), 150 mg/KgBW (n = 7), and 200 mg/KgBW (n = 6). Statistical analysis was performed using one-way ANOVA. Distinct lowercase letters indicate statistically significant differences ($p< 0.05$).

The glomerulus area slightly decreased in all treatment groups, with the 100 mg/kg BW group demonstrating the most noticeable decrease. The renal corpuscle area was similar between all groups; however, a slight decrease was noted in the 100 mg/kg BW group despite a minimal increase being observed in the two other treatment groups (Table 3 and Figure 2).

The glomerulus and renal corpuscle area ratio was significantly increased in the 100 mg/kg BW group ($p < 0.005$), whereas the control group and the other two treatment groups remained within the same range. In the 100 mg/kg BW dose, a slight increase in glomerulus area was accompanied by a decrease in corpuscle area, leading to an elevated ratio. This contrasts with the other treatment groups, where both glomerulus and corpuscle area increased proportionally, maintaining a constant ratio.

Table 3.
Renal glomerulus and corpuscle measurements.

Groups	Glomerulus Area (μm^2 ± SEM)	Renal Corpuscle Area (μm^2 ± SEM)	Glomerulus Area and Renal Corpuscle Area Ratio (± SEM)
Control	453.10 ± 19.34[a]	779.00 ± 42.07[a]	0.60 ± 0.03[a]
100 mg/KgBW	455.30 ± 9.58[a]	674.30 ± 34.97[a]	0.69 ± 0.02[b]
150 mg/KgBW	482.10 ± 20.59[a]	824.60 ± 56.75[a]	0.60 ± 0.03[a]
200 mg/KgBW	455.00 ± 25.43[a]	796.20 ± 71.71[a]	0.60 ± 0.02[a]

Source: Results were presented as mean ± SEM for control (n = 7), dosage 100 mg/Kg BW (n = 6), 150 mg/Kg BW (n = 7), and 200 mg/Kg BW (n = 6). Statistical analysis was performed using the Kruskal-Wallis test. Distinct lowercase letters indicate statistically significant differences ($p < 0.05$).

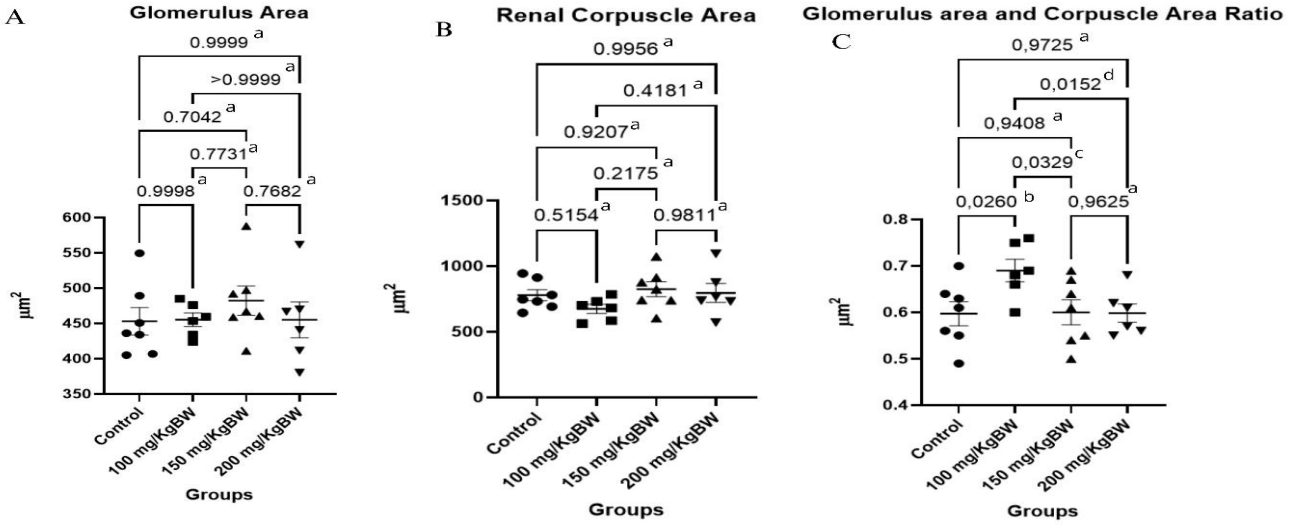

Figure 2.
Glomerulus area, renal corpuscle area, and their ratio, 53 days following the experiment, were conducted. The p-value between the two groups is presented above the bar. Different lowercase letters represent significant differences ($p < 0.05$). (n = 7, control; n = 6, 100 mg/Kg BW; n = 7, 150 mg/Kg BW; n = 6, 200 mg/Kg BW).

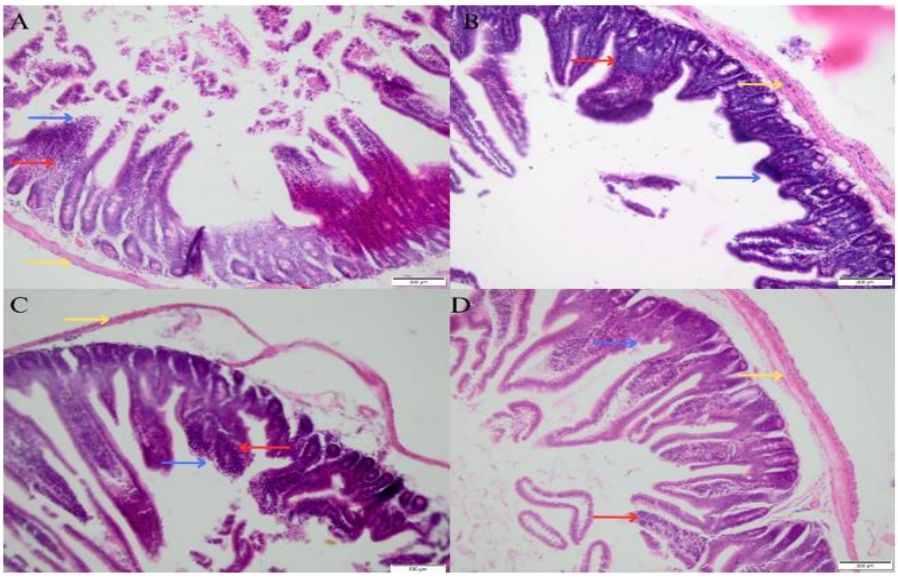

Figure 3.
Duodenum histology. A: control group, B: 100 mg/Kg BW, C: 150 mg/Kg BW, D: 200 mg/Kg BW. As shown in Figure B, the group receiving 100 mg/kg BW has the shortest villi compared to the other groups. The blue arrow shows ulceration, the red arrow points to inflammatory cells, and the yellow arrow shows the muscularis mucosa. HE Stain, 400x magnification.

Histological examination of the duodenum revealed that at a dose of 100 mg/kg BW, the duodenal villi exhibited significant shortening, accompanied by more pronounced ulceration. The surrounding muscularis mucosal layer appeared notably thinner. In contrast, at higher doses of 150 mg/kg BW and 200 mg/kg BW, the duodenal morphology demonstrated an improved appearance, closely resembling that of the control group Figure 3.

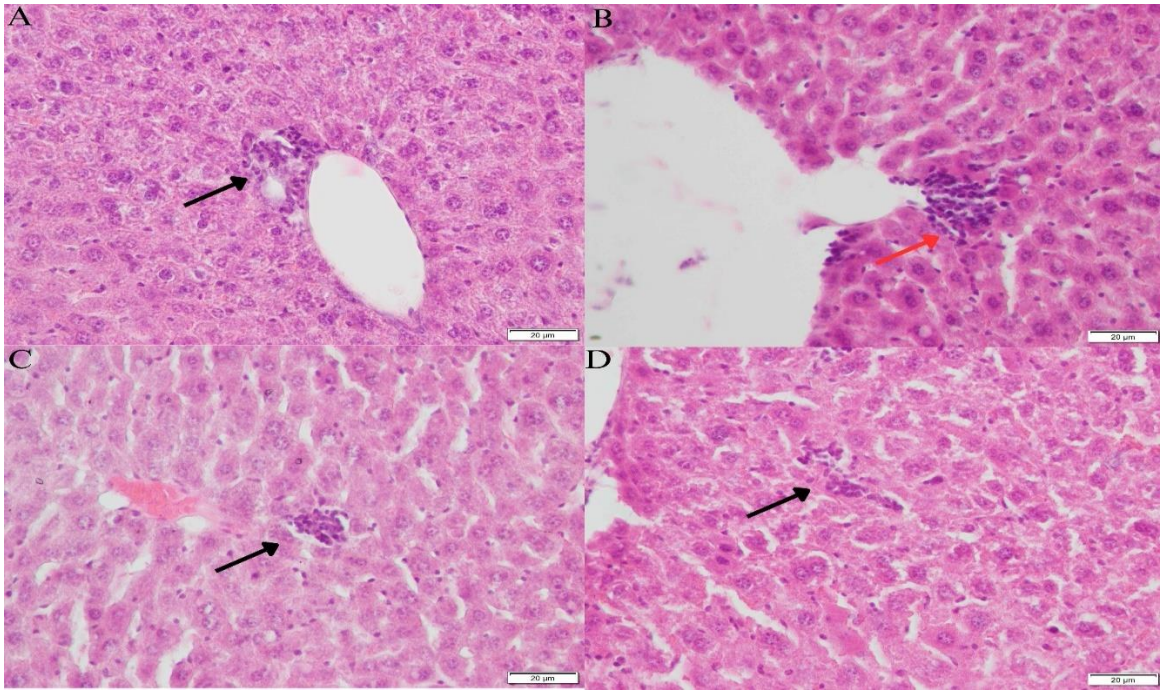

Figure 4.
Hepar histology of inflammatory cell infiltration. A: control group, B: 100 mg/Kg BW, C: 150 mg/Kg BW, D: 200 mg/Kg BW. As shown in Figure B, the group receiving 100 mg/kg BW has massive infiltration of inflammatory cells. The black arrow shows the infiltration of inflammatory cells, and the red arrow points to the massive infiltration of inflammatory cells. HE Stain, 400x magnification.

Histological examination of the hepar described that at a dose of 100 mg/kg BW, extensive infiltration of inflammatory cells occurred. In contrast, at higher doses of 150 mg/kg BW and 200 mg/kg BW, the hepar morphology exhibited improved performance, closely similar to that of the control group (Figure 4).

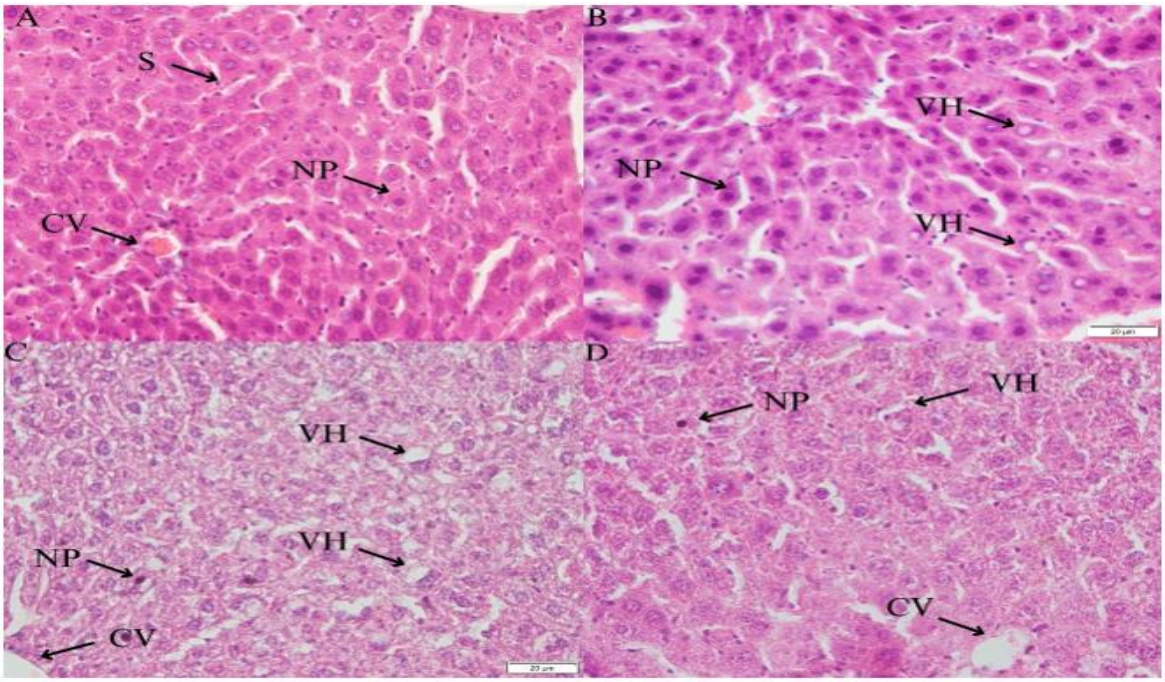

Figure 5.
Hepar histology. A: control group, B: 100 mg/Kg BW, C: 150 mg/Kg BW, D: 200 mg/Kg BW. CV: central vein, NH: normal hepatocyte with an open-faced type nucleus, arranged as a hepatic plate, S: Sinusoid, lined by endothelial cells, NP: Nuclear pycnotic, indicating hepatocyte degeneration, VH: Vacuolation of hepatocyte. HE Stain, 400x magnification.

The hepar histology showed alterations in hepar morphology, including vacuolation of hepatocytes and nuclear pyknosis (Figure 5).

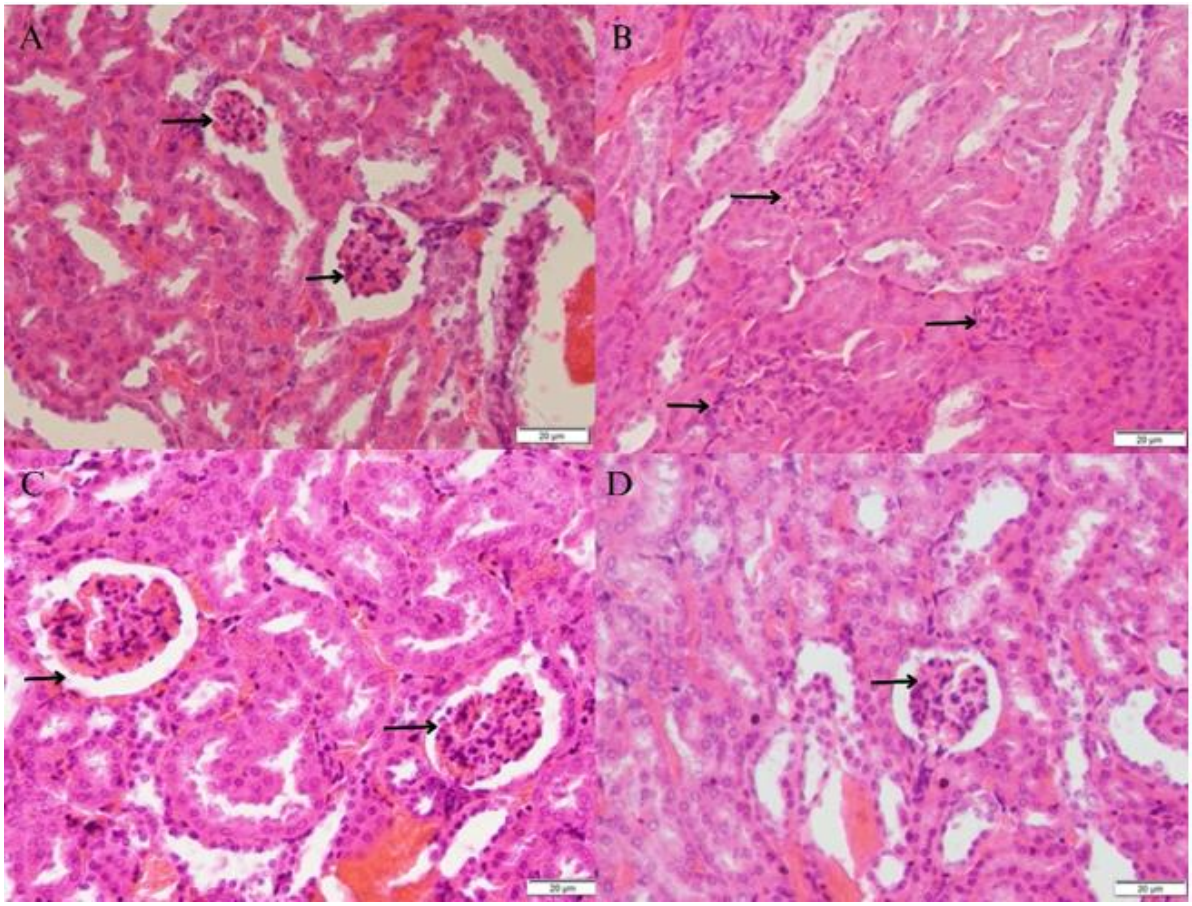

Figure 6.
Renal histology. A: control group, B: 100 mg/kg BW, C: 150 mg/kg BW, D: 200 mg/kg BW. The glomerulus (black arrow) in the cortex of the kidney shows an empty space surrounding it (Bowman's space), which is visibly narrowed or even absent in the group administered with 100 mg/kg BW of aluminum. HE stains, magnification 400x.

Renal histological analysis revealed alterations in the glomerular-to-renal corpuscle ratio across all treatment groups, with the most pronounced changes observed in the 100 mg/kg BW group.

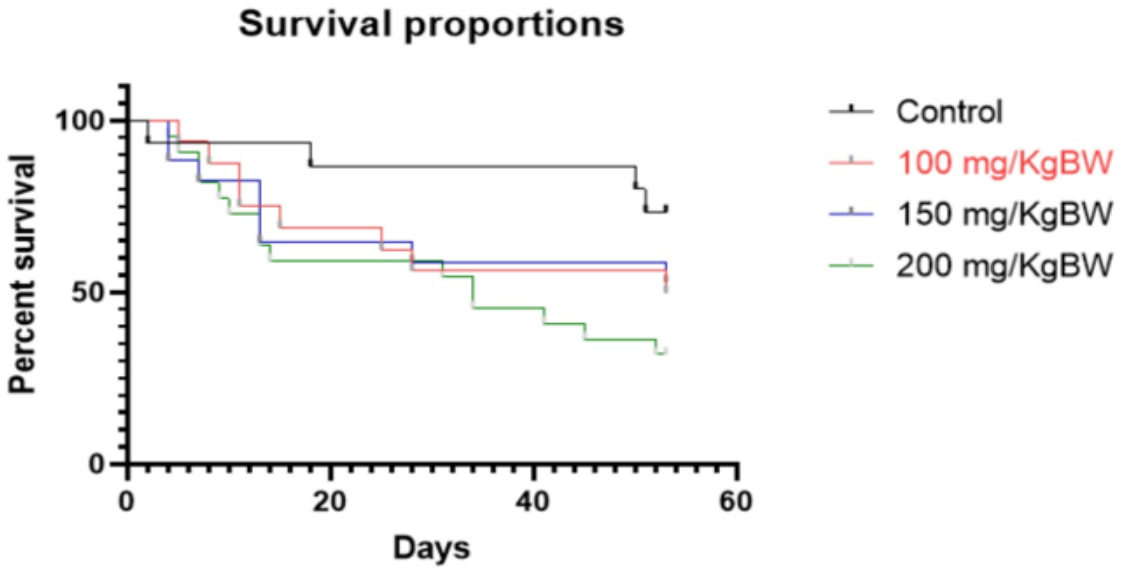

Figure 7.
The survival proportion showed that most mice deaths occurred during the first and second weeks; however, in group IV, deaths continued until the end of the experiment. The black line represented the control group, with a survival rate of 73%. The red line represented a treatment group with a dose of 100 mg/Kg BW, showing a survival rate of 53%. The blue line represented a treatment group with a dose of 150 mg/Kg BW, with a survival rate of 50%. The green line represented a treatment group with a dose of 200 mg/Kg BW, with a survival rate of 31%.

The Kaplan-Meier survival rate showed that the control group had better survival compared to all treatment groups. The worst survival was in the 200 mg/KgBW dose group, where 70% of the subjects died by the end of the experiment. The survival rates of the other two treatment groups were almost the same, with 50% of subjects dying. Deaths occurred throughout each week of treatment.

4. Discussion

Aluminium is known as a toxic agent affecting many organs [17]. This study explores the effects of aluminium via oral administration in the duodenum, liver, and kidneys. Aluminium was primarily filtered by the kidneys and detoxified in the liver via bile [17, 18]. The gastrointestinal (GI) tract is the major route for dietary aluminium. The duodenum is the main organ for absorbing dietary substances and facilitating their entry into systemic circulation, with its epithelial cells also serving as a barrier [19]. Histological analysis revealed differences in the infiltration of inflammatory cells and villi structure. This finding is in accordance with previous studies showing lymphocyte proliferation, a reduction in the number of goblet cells, and mucosal degeneration [20]. In this study, the lower dose showed more severe effects than the higher dose. A previous study on testes reported similar results, where the lower dose decreased sperm production and altered sperm morphology [4]. Studies on the brain also described similar findings, showing that lower doses of aluminium led to worse cognitive outcomes, increased oxidative stress in the hippocampus and prefrontal cortex, behavioral changes, and increased microglial activation compared to higher doses [21, 22].

Oral administration of Al at low doses induces visceral hypersensitivity. Mast cells and proteinase-activated receptor-2 (PAR2), as a major mediator of nociception, were upregulated. Al also induced persistent visceral pain, even after the cessation of treatment [23]. Al is absorbed through the gut at approximately 0.1% to 1%. The Al uptake through the gastrointestinal tract is complicated and affected by various factors, including age, pH, stomach contents, coexistence of substances, and the chemical speciation of aluminium. The intestinal barrier has been thought to be able to protect against aluminium due to the low bioavailability of Al in the gastrointestinal tract, suggesting that this tissue does not react adversely to aluminium [19].

Chronic Al administration led to shortened villus height and crypt depths, also reducing the ratio of increasing inflammatory markers, including interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 1β (IL-1β), and tumor necrosis factor alpha (TNF-α). Catalase activity, a biomarker for oxidative stress, was depleted, while lipid oxidation and protein levels were elevated [24, 25]. Tight junction proteins, such as claudin-1 and occludin, were decreased, disrupting the structural integrity of tight junctions, decreasing the regulation of permeability, and thus resulting in a variety of intestinal diseases [25].

The findings of this study focus on inflammation and nuclear degeneration of hepatocytes at the 100 mg/Kg BW dose, with no significant effects at higher doses. Arpita describes aluminum accumulation in the liver as dose-dependent, with the highest accumulation found at lower doses, similar to findings in the brain and the spleen, and it also caused DNA damage in those organs [26]. Previous studies have histologically demonstrated expanded portal tracts (EPT), congested central veins, dilated central veins (DCV), glomerular shrinkage, intense inflammatory cell infiltration, distorted sinusoids, and pycnotic nuclei [5, 7, 18, 25].

The liver function test parameters, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine transaminase (ALT), were elevated. ALP is an important marker of imbalance in the normal drainage of the biliary tree. Elevated ALT and AST levels are suggestive of hepatocyte dysfunction [7, 25]. AL also increases glucose, total protein, albumin, cholesterol, and triglycerides. Elevated glucose levels indicate disruption in glucose metabolism, which may be due to the inhibition of pancreatic B-cell activity and insufficient insulin secretion [7]. AL disrupts mitochondria in hepatocytes, thus altering the metabolism of carbohydrates, lipids, and amino acids and disrupting homeostasis [27].

This study shows elevated glomerular and corpuscle ratios in the 100 mg/KgBW group, but not in the higher doses, and is consistent with those observed in the duodenum and liver. These findings align with Sanai et al., which demonstrated that high doses of Al have a protective effect on renal dysfunction but are detrimental to nutritional status [28]. The glomerulus is an important component of the renal system that filters blood, including toxins. Several conditions may lead to glomerular damage, including drugs, infections, circulating factors, diabetes, inflammation, and toxins [29-31]. Al is one such toxicant that can cause renal damage. Al increases lipid peroxidation and oxidative stress, as well as oxidative stress to DNA and proteins. It decreases glutathione (GSH) content and glutathione peroxidase (GSH-Px), glutathione transferase (GST), and catalase (CAT) activities. It also alters renal-tubular p-aminohippuric acid transport, impairs sodium-water balance, and renal-tubular phosphate reabsorption. It inhibits Na+/K+ ATPase activity, leading to increased free intracellular Fe+2, which results in further oxidative stress [17].

Previous studies have described the effects of Al on the renal system. Rizkawati et al. showed the effects in mice of different ages, including glomerular atrophy, blood-filled spaces, disintegration of renal tubular epithelium, interstitial fibrosis, and robust mesenchyme in the glomeruli. Younger renal mice are more vulnerable to damage [18]. Immunohistochemistry staining showed strong expression of Ki-67 and P5 [32]. Renal function measurements, including AST, ALT, creatinine, and urea, were increased [12, 33, 34]. Antioxidant enzyme activity, including superoxide dismutase (SOD), CAT, GSH-PX, and GSH, was depleted, while malondialdehyde (MDA) levels were elevated [9, 10, 33].

Al toxicity affects the survival rate. Aluminum intake accumulates in the body and causes toxic effects. These effects cause systemic toxicosis in various organs. Aluminum accumulation depends on its route of exposure; inhalation and ingestion are the main routes. Toxic effects of aluminum can cause degeneration, apoptosis, necrosis, atrophy, and dysplasia in cells [3]. Aluminum's pro-oxidant activity, which leads to oxidative stress, free radical damage, and oxidation of cellular proteins and lipids, is primarily responsible for its toxic effects. This activity is considerable at quantities of aluminum that are typically found throughout the body [35].

This study describes the histology of the duodenum, liver, and kidneys in the 100 mg/KgBW group, which showed the most significant alterations compared to the other groups, although these changes were not correlated with the survival rate. The findings propose several factors influencing the mice's survival. Aluminum accumulation varied across organs, with the highest accumulation found in the brain, followed by the liver, serum, and kidneys [36]. The gradual deaths showed a progressive toxic effect. Deaths in the control group were unrelated to treatment but were instead caused by stress, as animals experienced stress due to changes in cages, environment, and feeding patterns Morais, et al. [37] as well as food scrambling and fighting. Aluminum toxicity has been linked to pro-oxidant activity across various organs and tissues. The total antioxidant capacity, which plays a role in cellular defense and protection against elevated oxidative stress, varied among aluminum exposure models depending on the organ examined. For instance, exposure to low doses of aluminum (1.5 and 8.3 mg/kg BW/day) reduced antioxidant capacity in the testis, whereas higher doses resulted in an increased antioxidant profile [4]. Neurotoxic effects of aluminum at lower doses may occur more than at higher doses [38].

This non-linear dose-response relationship may be attributed to the size of aluminum agglomerates, surface charge, and stability, which influence cellular uptake and transport mechanisms. The traditional principle of "the dose makes the poison" is overly simplistic, particularly in the context of endocrine disruptors. These findings underscore the need for a critical reassessment of current toxicological methodologies used to establish safety standards, especially for substances like aluminum, which may elicit complex and non-linear effects within biological systems [26, 39-41]. Furthermore, the toxicity of aluminum is contingent upon exposure circumstances, individual and intrinsic characteristics, and the resulting distribution and bioavailability within the body [4].

The limitations of this study include the failure to investigate other potentially affected organs, the absence of an assessment of organ function, and the lack of aluminum accumulation analysis within the tissues.

5. Conclusion

Aluminium, a heavy metal widely used in daily human activities, exerts negative effects on various organs. Al can enter the body through multiple routes, including oral exposure. This study investigates the effects of Al on the histological features of the duodenum, liver (hepar), and kidneys at different doses. Duodenal histological alterations were characterized by villous shortening and infiltration of inflammatory cells. In the liver, inflammatory cell infiltration and nuclear degeneration were observed. Renal histology revealed alterations in the glomerular and corpuscular structures. Interestingly, the most severe histopathological changes were observed at the lowest dose.

This nonlinear dose-response finding suggests an adaptive mechanism to Al exposure. Although discrepancies exist among previous studies, some findings align with the results of this study. Given the limited evidence supporting this hypothesis, further investigations are warranted to elucidate the underlying mechanisms, including biomolecular analyses and determination of critical doses.

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