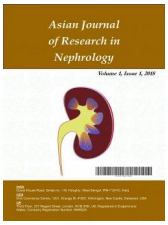

*Asian Journal of Research in Nephrology*

*2(1): 1-9, 2019; Article no.AJRN.47450*

# Some Morphological and Biochemical Changes in the Kidney of Adult Wistar Rats Following Aluminium Chloride Exposures

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author AJA designed the study, performed the statistical analysis. Author BDK wrote the protocol and wrote the first draft of the manuscript. Author AAA managed the analyses of the study. Author OOA managed the literature searches. All authors read and approved the final manuscript*

***Article Information***

Editor(s):
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(3) Idorenyin Umoh, University of Uyo, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/47450

***Original Research Article***

***Received 29 November 2018***
***Accepted 27 February 2019***
***Published 30 March 2019***

## ABSTRACT

In modern life, exposure to aluminium is inevitable as it is released primarily from natural process. Humans are exposed to aluminium through food packaging, medicines, water and air. Kidney is a urinary organ which eliminates most toxins and other foreign substances that are either produced by the body or ingested into the body.

The present study investigated some of the effects of Aluminium chloride on the kidney of adult wistar rats. Thirty two (32) rats of both sexes were used for this study and were separated into four groups based on their gender. The rats in group A (8 rats) were regarded as the control and they received only distilled water and stock diet throughout the period of experiment. The rats in group B, C and D orally received Aluminium chloride at 500mg, 1000mg and 1500 mg/kg respectively for 31 days. The rats were sacrificed on the 32nd day by cervical dislocation. The trunk of each rat was

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dissected and blood was collected from the heart for biochemical evaluation. The kidneys were removed, rinsed and weighed before fixing in 10% formal saline for histological studies using H&E staining techniques.
Morphometric study shows significant differences in the organ weights between kidney weights in control and aluminium-treated rats. Kidney weights of aluminium-treated rats were significantly reduced (P<0.05) compared with controls. The decrease was significant (P<0.05) in both kidneys in group B and in the left kidney for group D.
The biochemical evaluation also showed significant increase (P<0.05) in alkaline phosphatase (ALP), urea and creatinine level in Aluminium- treated groups.
The histological results showed architectural disarrangement, including degenerative changes and dilatation and distortion of proximal tubule which increased in a dose-dependent manner in experimental groups.
This study concluded that Aluminium chloride have adversely affected the histology of the kidney in treated wistar rats which may invariably result in impairment of renal function.

*Keywords: Aluminium; toxicity; histology; kidney; degeneration; creatinine; tubules.*

## 1. INTRODUCTION

Concern has been raised about the neurotoxicity of aluminum chloride as it is involved in the atieology of certain diseases such as dementia, Alzheimer disease parkinsonism and amyotrophic lateral sclerosis [1]. The adverse effects have been well documented on the brain tissue bone; liver, blood cells and kidney [2-3]. Previous study has shown that Aluminum chloride accumulatses in the kidney and induces renal degeneration in kidney tabular cells, leading to nephrotoxicity [4-6].

Similarly, it has been reported that salt of aluminum has the tendency to bind RNA and DNA thereby inhibiting some activities of enzymes such as acid and alkaline phosphatases, phophoxydase, phosphodi-esterase and hexokinase [7].

Aluminum has been described as the most abundantly available metal in the earth as its production and use are enormous compared with any other heavy metallic substance [8]. Aluminum is extensively used in canning, tanning, building, automobile, aviation, paper, paint, ceramic and glassware industries [8]. Aluminium and its salts were formerly considered to be non toxic and so its use in medicine increased significantly. Aluminium magnesium silicate and Aluminium hydroxide are used as antacid and Aluminium gel as phosphate binding agent to reduce plasma phosphorus level in patients suffering from acute renal failure [8]. It has been reported that aluminum intake in such patients is approximately between 800 to 5000 mg / day [8], which is far above the provisional WHO tolerable weekly intake of Aluminium (PTWI). WHO has stated that the PTWI of Aluminium as a contaminant is 7 mg/kg body weight for adults, while acceptable daily intake (ADI) for children is considered to be 2 mg/kg body weight [9].

Furthermore, previous studies have implicated aluminum as a potential occupational toxin that is known for more than five decades [8]. Investigation has shown that one of the 0primary exposure of Aluminum is through diet, in addition to environmental and occupational exposures. Similarly, as a result of the wide use of Aluminium as cookware and storage vessels, the exposure and intake of aluminum by Indian population is much higher that what has been reported for the western part of the world [8]. Report from previous studies has indicated that the kidney is one of the main organs adversely affected by indiscriminative exposure to aluminium [10-11]

Aluminium is the most abundant metallic element and the third most prevalent element (comprising approximately 8%) exceeded only by oxygen (47%) and silicon (28%) in the earth's crust [12].

The elemental aluminium does not occur in its pure state but it is always combined with other elements such as chloride, hydroxide, silicate, sulphate and phosphate. The wide distribution of this element makes its exposure to be virtually inescapable [12].

Annual production of aluminium is about 22,000 metric tonnes worldwide [13]. It has been noted that inhalation of the fine particles of aluminium metal dust in factories cause both encephalopathy and pulmonary fibrosis in human

beings. Aluminium has also been implicated in neurotoxicity associated with amyotrophic lateral sclerosis [14] and Alzheimer's disease [15] in indigenous population of Guam where soil have Aluminium as high as 150-600 gm/kg [16]. One of the main exposures of aluminium is through diet in addition to environment and occupation. Because of the wide use of Aluminium cookware and storage vessels, the intake of aluminium by India population is much higher than what has been reported for the West. One of the main organs affected by Aluminium ingestion is kidney **[**10-11].

Human exposure to aluminium comes from food, drinking water, pharmaceuticals as well as from the environment [17]. The normal average daily intake is 1 to 10mg for adults [17]. Aluminium is poorly absorbed following either oral or inhalation exposure and is essentially not absorbed dermally [18]. Report has revealed that it is removed from blood by the kidneys and excreted in urine [18]. Aluminium is widely distributed in the environment and extensively used in daily life, which causes its easy exposure to human beings [19]. It gets access to the human and animals' body via the gastrointestinal and the respiratory tracts [19].

However, Aluminium is present in our water, foods, medications and air. The healthy human body has effective barriers such as skin, lungs, and gastrointestinal tracts, against aluminium Furthermore, most of the aluminium in natural plant foods is bound with other substances such as Silicon which prevents absorption of the Aluminium into the body. The harmful (unbound, more absorbable) forms of Aluminium enter our foods additives such as leaving agent and emulsion [20]. This present study assessed the histopathological and biochemical changes in the kidneys of wistar rats following Aluminium chloride exposure.

## 2. MATERIALS AND METHODS

Thirty-two wistar rats of both sexes with an average weight of 140gms were used for the present studies. The rats were procured from miracle farm Ogbomosho. The rules and regulations governing animal handling were strictly observed. The wistar rats were kept for two weeks before commencement of aluminium chloride administration. This was done in order to allow them get adapted to the environment. They were housed in standard polypropylene cages in an environmentally controlled well ventilated room and photoperiod of 12 hours daily. No artificial light was used. The rats were maintained under standard laboratory conditions. They were also given adequate care in accordance with the principles of laboratory and animal care as indicated and published by Institute of Laboratory Animal Resources 1996 [21]

The rats were given adequate feed (grower mash) and tap water *ad libitum*. The feed was bought from Bova Jay at Orita-naira, Ogbomosho and its constituents are: maize, soya meal, ground nut cake (GNC), wheat offal, corn brain, palm kernel cake (PKC), fish meal 65% (local fish), bone meal, oyster shell or limestone. Vitamin & minerals (premix), growers, lysine, methionine and salt.

Contact bedding (Wood shaving) was used in the bottom of the cages, in order to allow the animals to form their own microenvironment.

Aluminum chloride salt was purchased from the chemical supplier from Ilorin, Kwara State. Distill water was bought from the department of Food Science and Engineering, LAUTECH. 50g of Aluminium chloride salt was dissolved in 100ml of distill water. The solution was shaken until the salts were completely dissolved. Stock solution was prepared and kept inside a refrigerator.

### 2.1 Experimental Design and Grouping

The experimental animals were weighed before acclimatization. After acclimatization, the wistar rats are reweighed and are randomly divided into four groups based on their gender to prevent them from mating. The groups were treated as follows:

Group A was the control group and they were given stock diet and distill water only.

Group B Wistar rats were given stock diet and aluminium chloride at a dose of 500 mg/kg orally for one month (31 days).

Group C wistar rats were given stock diet and aluminium chloride at a dose of 1000 mg/kg body weight which is equivalent to 0.32 ml/day (intermediate dose) orally for one month (31 days).

Group D rats were given stock diet and aluminium chloride at a dose of 1500mg/kg body weight which is equivalent to 0.42 ml/day (high dose) orally for one month (31 days).

The intragastric administration was done with the use of cannula and attached calibrated syringe in experimental animals.

The animals were weighed after the last dose was administered before sacrifice. They were sacrificed by cervical dislocation on the 32nd day. Blood were collected from the heart for biochemical analysis of enzymes and the tissues (kidney) were harvested immediately for histological analysis after aluminium chloride had been administered for 31 days. The kidneys from each of the groups were weighed with the use of sensitive scale and fixed separately in 10% formal saline.

The kidneys of each rat from each group was removed, weighed and fixed in 10% formol saline

## 2.2 Statistical Analysis

Experimental results were analyzed and tested for significance using unpaired student t-test in graph pad prism version 6.0. If P value of the t-test is less than 0.05 (P<0.05), then the result is significant. If P value is greater than 0.05 (P>0.05), it implies that the result is not significant.

# 3. RESULTS

## 3.1 Effects of Aluminium Chloride on the Kidney Weights

Table 1 below shows relative organ weights following administration of aluminium on the rats in the treated groups with those in the control group. There was significant decrease in the dry weights of right and left kidneys in group B and left kidney in group D as compared with the control group ($P < 0.05$), while others showed no significant decrease as compared with the control group ($P > 0.05$). In the experimental studies, the rats were sacrificed by cervical dislocation a day after the administration ended (32nd day). There was no morphological change observed after the kidneys were harvested and physically examined by comparing the experimental with the control group. After the kidneys were blotted dry and weighed, it was discovered that there was significant decrease in the dry weights of right and left kidneys in group B and left kidney in group D as compared with the control group, while others show no significant decrease as compared with the control group as shown in Table 1.

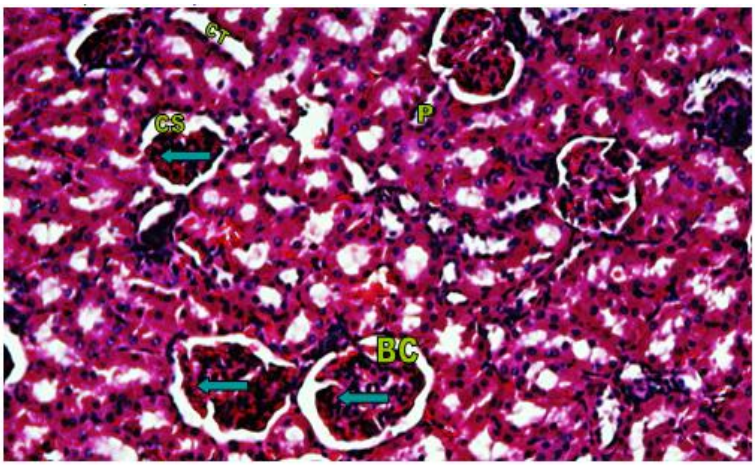


**Plate 1. Transverse section of the rat's kidney in group A (H&E X100)**

**Table 1. Mean ± S.E.M of the dry weights (g) of the kidneys after the administration of Aluminium chloride**

| Groups | Left kidney (g) | Relative organ weight (%) | Right kidney (g) | Relative organ weight (%) |
|---|---|---|---|---|
| A | 0.65 ± 0.02 | 0.39 | 0.57 ± 0.02 | 0.34 |
| B | 0.47 ± 0.03* | 0.34 | 0.44 ± 0.02* | 0.32 |
| C | 0.58 ± 0.04 | 0.36 | 0.53 ± 0.04 | 0.33 |
| D | 0.53 ± 0.08* | 0.32 | 0.48 ± 0.08 | 0.29 |

*Dry weights of the rats are expressed as Mean ± S.E.M using student t-test. Significance: P<0.05 (*). Values less than 0.05 were considered significant while values greater than 0.05 were considered insignificant*

### 3.2 Biochemical Evaluation

Table 2 below shows the analysis of biochemical parameters in kidneys of adult wistar rats after administration of aluminium for four weeks.

When the parameters measured were compared intreated groups (group B, C and D) with that of control group (group A), it could be seen that there was significant increase in ALP (alkaline phosphatase), urea and creatinine level in group B, C and D ($P < 0.05$) aluminium-treated group. The increase occurred in a dose dependent – manner in all the parameters as shown in the Table below.

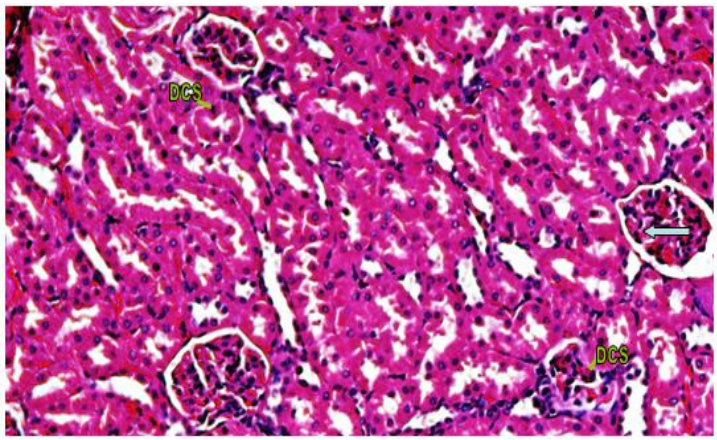


**Plate 2. Transverse section of the kidney of the rats in group B after administration of 500 mg of aluminium chloride for four weeks. (H&E X100). This photomicrograph shows mild disarrangement of kidney architecture with decreased capsular space (DCS) and mild degeneration of glomerulus (arrow)**

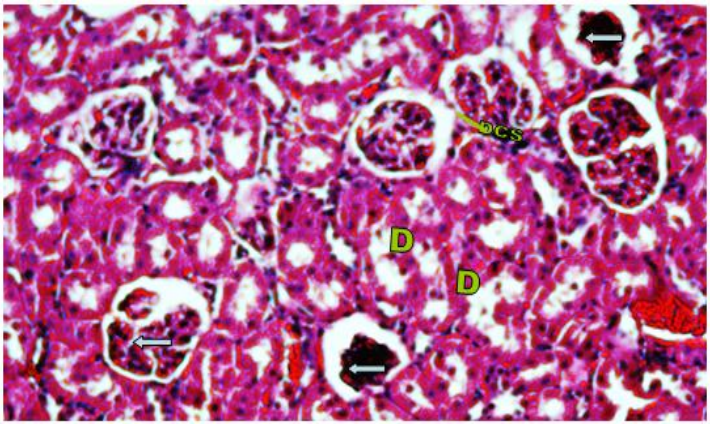


**Plate 3. Transverse section of the kidney of the rats in group C after administration of 1000mg of Aluminium chloride for four weeks (H&E X100)**

**Table 2. Mean ± S.E.M of serum analysis of the kidney after administration of Aluminium chloride**

| Biochemical paramaters | Group A (Control) | Group B (LD) | Group C (MD) | Group D (HD) |
|---|---|---|---|---|
| ALP | 3.734 ± 0.51 | 6.981± 0.73** | 9.416± 0.48**** | 11.36± 0.44**** |
| UREA | 5.725 ± 0.37 | 7.400± 0.23** | 9.325± 0.69** | 13.13± 0.46**** |
| CREATININE | 55.25 ± 1.44 | 87.50± 4.41*** | 106.5± 4.57**** | 120.5± 2.87**** |

*The t-test table of the Mean ± S.E.M of the biochemical parameters of kidney after administration of aluminium chloride is shown above. Significance: P<0.05 (*)*

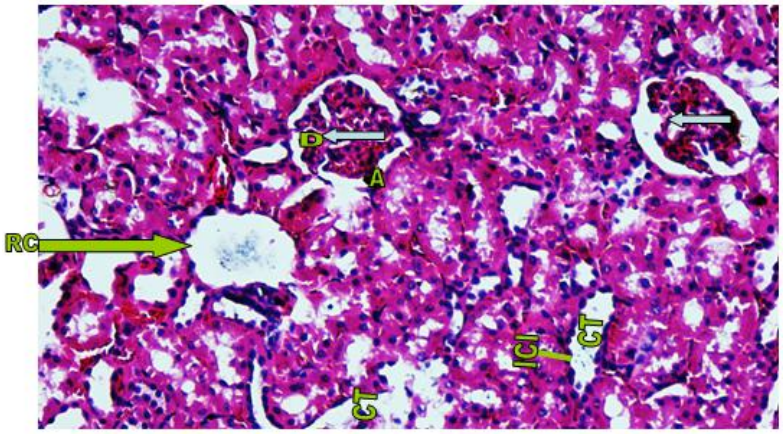


**Plate 4. Transverse section of the kidney of the rats in group D (H&E X100) after administration of 1500mg of Aluminium chloride for four weeks. The architecture of the kidney is grossly disarranged with severe degeneration of glomerulus (blue arrow). The renal corpuscles (RC) appeared degenerated, dilatation of collecting tubules with inflammatory cell infiltration (ICI) were prominent**

The above micrograph shows (Plate 1) the transverse section of kidney in the control .The renal cortex which contains normal glomerulus (arrow) enveloped by visceral layer (VL) of Bowman's capsule (BC). The capsular space (CS) is also well-defined. The parietal layer (PL) of BC is lined by simple squamous epithelium. All the renal structures appeared normal.

The architecture of the kidney is disarranged. There was also degeneration of glomerulus (arrow), decreased capsular space (DCS), distortion of Bowman's capsule (BC) and dilatation of proximal tubule (DPT) with loss of urinary pole (Plate 3).

## 4. DISCUSSION

Aluminum (Al) is ubiquitous in the environment and exposure to it is unavoidable [22]. It has been described as a serious environmental toxicant and included in the priority list of hazardous substances identified by Agency for Toxic Substances and Disease [23] Registry Increased attention is being focused on possible adverse effects of aluminium on human health. Aluminum (Al) is ubiquitous in the environment and exposure to it is unavoidable [22]. It is a serious environmental toxicant and included in the priority list of hazardous substances identified by Agency for Toxic Substances and Disease Registry [23]. Human exposure to aluminium is from its natural occurrence in the environment i.e. through food, water and air as well as from aluminium deliberately introduced into the environment by man. Aluminium compounds are used in pharmaceuticals (antacids, analgesics, antiperspirants) in water treatment processes (as coagulant) and as metal in consumer products. Aluminium is present in virtually all plants. Foods naturally high in aluminium include potatoes, spinach and tea. Processed dairy products, flour and infant formula may be high in aluminium, if they contain aluminium compounds as food additives [24].

The present study was designed to investigate the effects of oral administration of aluminium chloride on the kidney based on morphometrical, histological and biochemical approach for four weeks using adult wistar rats of variable weights. Findings from previous study has indicated a well marked dose dependent morphological changes in the kidney tissue which became prominent in the proximal tubules following administration of 20 mg/kg of aluminium chloride [25]. The result of this study is also in agreement with the report of similar study carried out on rats that were treated with aluminium nitritotriacetic acid compound which resulted in severe renal damage with proximal tubular degeneration of renal tissue [26].

The rats were weighed at regular interval and there was significant decrease in body weight of group C and D in week 1, group B also showed significant decrease in week 2 and week 4, while others showed no significant decrease as compared to group A as shown in Table 1 in chapter four. This is because toxic aluminium a

may decrease feed intake by modulation of appetite. In general sense, any substance which disturbs metabolism or physiology sufficiently to cause growth inhibition causes decreased feed intake. These substances also inhibit ATP production and therefore inhibit protein and active transport, with a final result of decreased body weight and feed intake. The decrease in body weights was also supported by previous report [27] where; mean value indicated that treatment with aluminium chloride caused significant decrease in body weight. During their 3 months observation, rats receiving aluminium chloride decrease in water and food intake, which result in decrease final body mass.

In the present study, biochemical evaluation shows significant increase in ALP (alkaline phosphatase), urea and creatinine level after the toxicant exposure ($P < 0.05$) as shown in Table 2. The result of this study was supported by previous report of [28], who found that after the aluminium exposure the level of urea and creatinine were significantly increased, because of kidney dysfunction. The increase of blood urea and serum creatinine concentration can be a consequence of critical accumulation of this metal in kidney and following renal failure development as aluminium is excreted mainly by kidney [29].

The significant increase in serum urea following the administration of the $Alcl_3$ may be due to increased protein catabolism or renal dysfunction [30].

The measurement of the activities of enzymes in tissues and body fluids plays a significant and well-known role in disease investigation and diagnosis which were reported [31], assault on the organs/tissues and to a reasonable extent the toxicity of the drug [31].

Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by the $Alcl_3$. Alkaline phosphatase is a "*marker*" enzyme for the plasma membrane and endoplasmic reticulum. The increased enzyme activity might have resulted from increased functional activity of the tissues caused by Aluminium [32]. It is often employed to assess the integrity of plasma membrane and endoplasmic reticulum [33].

The observed significant increase in all the parameters investigated is an indication that the compound has manifested its deleterious effect on the renal tissue significantly. This may probably be due to the defense system of the animals which must been compromised by the administered compound.

Similarly, measurement of aluminium levels in plasma and urine by atomic absorption spectroscopy and aluminium levels were also carried out in kidney at cellular and subcellular levels by electron probe X-ray microanalysis (EPXMA). These authors have found significant amount of aluminium in cytoplasm and mitochondria in proximal convoluted tubules of kidney whereas raised levels were not detected in the control animals. This is indicative of concentration of aluminium in cellular organelles leading to structural and biochemical changes at subcellular level.

The present histoloical examination for the transverse section of kidney in the control group (group A) which shows normal glomerulus enveloped by visceral layer of Bowman's capsule. The capsular space is also well-defined. The parietal layer of BC was lined by simple squamous epithelium. The arterioles were also present with normal morphology. The distal tubule, macula densa, juxtaglomerular cells, and proximal tubule are morphologically normal.

In the experimental groups, various histopathological changes have been observed in kidney tissue in the present study. General architectural disarrangement, including degenerative changes, decreased capsular space, dilatation of collecting tubules with inflammatory cell infiltration and dilatation of proximal tubule which increased with increase in administration of $Alcl_3$ has been observed in kidney tissue.

Reports from findings from previous works are in consistent with the histopathological changes observed in the present study. In addition to above findings, inflammatory cell infiltration, thickened basement membrane and decreased Bowman's space at places was observed in the present study. Disarrangement in kidney architecture like inflammatory cell infiltration, decreased capsular space, dilatation of proximal tubule and degeneration of the glomeruli have been observed in kidney of wistar rats in this work which was much in the groups that received 1500 mg/kg body weight (high dose) after

administration of aluminium chloride orally for 31 days Inflammatory cell infiltration, thickened basement membrane and decreased Bowman's space observed in the present studies which are in correlation with previous report [8]. It is possible that the architectural derangement of kidney cells observed in the present study may be due to altered and distorted cellular organelles like mitochondria, endoplasmic reticulum, lysosomes and cell membrane by aluminium.

This study concluded that aluminium chloride exposure had detrimental effects on the histology of the kidneys of wistar rats which invariably may result in a compromise of renal function. Further research should be carried out on the toxicological effects of this compound on different visceral of rats over an extended period of time to corroborate this report

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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