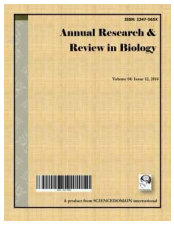

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Effects of Deionization Water Treated with Different Dose of Aluminum Chloride ($AlCl_3$) on Creatinine and Liver Enzymes of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EIS performed experiments. Author MKS supervised experiments, assisted in manuscript preparation and managed analysis the results. Author SIYA conceived the idea and supervise. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was done in Department of Biochemistry and Molecular Biology. Aluminum chloride is used as coagulant for treatment the drinking water in AlMogran Water Treatment Plant, Khartoum State, Sudan. The study aimed at assessing the concentrations of liver enzymes (AST and ALT) and creatinine of Wister rats treated orally with Aluminum chloride by using deionization water. Fifty adults rats (average body weight about 109 g) were divided into five groups (10 male per group) as follows Group one (G_1) represented control (without treatment), Group two (G_2) received tap water, Group three (G_3) received 50 mg/kg/day deionizing water treated with $AlCl_3$, Group four (G_4) received 60 mg/kg/day deionizing water treated with dose of $AlCl_3$ and Group five (G_5) received 70 mg/kg/day deionizing water treated with dose of $AlCl_3$.

The treatments were given orally by gavages and continued daily for 60 days. Then blood sample was collected from each rat and measured for liver enzymes and kidney creatinine. The results showed that $AlCl_3$ had led to a significant increase in liver enzymes and kidney creatinine ($P \leq 0.05$).

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In addition histopathology of liver of rats in G_4 (60 mg \ kg Al C_3) was showed severe necrosis, while the kidney showed packing, dilatation of renal tubules and degeneration compared with control group.

Keywords: Aluminum chloride; liver enzymes; plasma creatinine and wistar rats.

1. INTRODUCTION

Aliminum chloride is used in either anhydrous or hydrated form. In the anhydrous form, it is used as a catalyst, in Friedel-Crafts reactions, in the manufacture of rubber, the cracking of petroleum and the manufacture of lubricants. In its hydrated form, it is used by the pharmaceutical industry as an active ingredient in deodorants and antiperspirants, as well as in wood preservation, and in the manufacture of adhesives, paint pigments, resins, fertilizers and astringents [1,2,3]. Aliminum Chloride is used as chemical intermediate for Aliminum compounds and as coagulant in drinking water. Aliminum chloride was said to have negative effects on behavioral endpoints of Wister rats (alters behavior) [4].

Kidney plays major role in preventing accumulation of Aluminum ion by excreting out through urine [5]. Aliminum ion accumulation in kidney promotes degeneration in renal tubular cells, inducing nephrotoxicity [6]. The main route of Aluminum ion excretion is the urine; therefore, subjects with kidney malfunction or immature kidney, such as nephropathy patients or neonates, might experience toxic accumulation of Aliminum ion in the body [7]. Aliminum ion has the potential to be toxic for both human and animals. It was included in the priority list of hazardous substances identified by The Agency for Toxic Substances and Disease Registry [8]. It accumulates in various mammalian tissues such as the kidneys, liver, heart, brain and is related to cardiotoxicity, nephrotoxicity, neurotoxicity and hepatic dysfunctions [9].

2. MATERIALS AND METHODS

2.1 Experimental Protocol

Fifty adults Wister rats males weighting about 109 grams were kept under standard conditions (Temperature 26±3℃; humidity = 40 – 50%; 12:12 h light: dark cycle) in a well ventilated animal house, which was the source of experimental animals. Following adaptation period of 10 days, rats were fed with standard chow and free tap water when they were out of metabolic cage. The rats were randomly divided into five (40 rats \group): group one (G1) served as control (received deionizable water, 0.0 mg/kg/day$AlCl_3$) while group two (G2) was treated with tap water, group three (G3) treated with 50 mg/kg/day $AlCl_3$ group four (G4) treated with 60 mg/kg/day$AlCl_3$ and group five (G5) treated with 70 mg/kg/day$AlCl_3$. Treatment of deionizing water for experimental animal in present study was done orally administration with various concentrations of Aliminum chloride to each group of the Wistar rats. At end of each experiment Wistar rats were anesthetized after 12 hours fasting. Whole blood samples were collected from the hepatic portal veins at two tubes. Bloods of the first tubes were left for 15 minutes at room temperature then centrifuged at 4000 rpm for 20 minutes for separation of the serum which were kept in plastic vials at −26± 3℃ until analysis. For ethical considerations, the collection was done from Wister rats and with the permission of Department of Scientific Research, Al-Neelain University.

2.2 Estimation of Aspartate Transaminase (AST)

It was determined according to [10].

2.2.1 Principle of the methods

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxaloglutarate, forming oxaloacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm; by means of the Malate Dehydrogenase (MDH) coupled reaction.

$$\text{Aspartate} + 2\ \text{Oxaloglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{Glutamate}$$

$$\text{Oxaloacetate} + H^+ + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

2.2.2 Procedure

The working reagent and the photometer were brought to 37℃. The working reagent was pipetted into a cuvette. Then solution was mixed and the cuvette was inserted into the

photometer. Start stop watch, then after one minute the initial absorbance reading was recorded at one minute intervals. Thereafter for three minutes, the difference between consecutive absorbance readings were calculated and an absorbance difference per minute (ΔA/min) was averaged.

2.2.3 Calculations

The AT (GOT) concentration in the sample is calculated by using the following formula

$$U\backslash l = \frac{\Delta A\backslash \min X Vt X 10^6}{l X Vs X \xi}$$

Where:

ξ=Molar absorbance of NADH at 340nm (=3600), 1 =Light path (One1cm), Vt = Total volume and Vs = Sample volume.

2.3 Estimation of Alanine Aminotransferase (ALT)

It was determined according to [11].

2.3.1 Procedure

The working reagent and the photometer were brought to 37℃. The working reagent was pipetted into a cuvette. It was mix and then cuvette was inserted into the photometer .Start stop watch, then after one minute the initial absorbance reading was recorded at one minute intervals. Thereafter for three minutes, calculate the difference between consecutive absorbance readings were calculate and the absorbance difference per minute (ΔA/min) was averaged

2.3.2 Principle of the methods

Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from alanine to 2-Oxoglutarate, forming pyruvate and glutamate .The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm by mean of the lactate dehydrogenase (LDH) coupled reaction.

$$\text{Alanine} + 2\text{Oxoglutarate} \xrightarrow{ALT} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{LDH} \text{Lactate} + NAD^+$$

2.3.3 Calculations

The ALT concentration in the sample is calculated by using the following general formula

$$U\backslash l = \frac{\Delta A\backslash \min X Vt X 10^6}{l X Vs X \xi}$$

Where

ξ=Molar absorbance of NADH at 340nm (=3600), 1 =Light path (One1cm), Vt = Total volume and Vs = Sample volume

2.4 Estimation of Plasma Creatinine

It was determined according to [12].

2.4.1 Procedure

The working reagent and the photometer were brought to 37℃. Pipette the working reagent into a cuvette. Mix and insert cuvette into the photometer. Start stop watch, then after one minute record initial absorbance at one minute intervals. Record the absorbance at 500 nm after 30seconds (A_1) and after 90 seconds (A_2). Blood samples were collected at slaughter in plane tubes and centrifuge immediately at 3500 rpm for five min and analyzed for the activities of creatinine concentrations by using (BioSystem, S.A, Costa Brava 30, Barcelona. Spain 2011).

2.4.2 Principle of the method

Creatinine in the sample react with picrate in alkaline medium forming colored complex. The complex formation rate is measured in a short period to avoid interferences

2.4.5 Calculations

The creatinine concentration in the samples is calculated by using the following formula:

$$C\ sample = \frac{(A_2 - A_1)\ sample\ X\ C\ standard\ X\ dilution\ factor}{(A_2 - A_1)\ standard}$$

2.5 Statistical Analysis

Data from, aluminum ion concentration were analyzed using Statistical Package for Social Science (SPSS).

3. RESULTS AND DISCUSSION

Table I indicated that the values of Creatinine level for G_1, G_2, G_3, G_4 and G_5 were 0.50, 0.62, 0.70, 0.96 and 1.40 U/L, respectively. In analysis of variance, there was significant difference between group G_3 (0.7), G_4 (0,96) and G_5 (1.40) at ($P \leq 0.01$ whereas there was no significant

difference between group G_1(0.5) and G_2 (0.62). These results indicated that creatinine of treated deionizing water with Aliminum chloride was increased with increasing the dose of AlCl3 in deionizing water compared with G_2 (Tap water) and G_1 (deionizing water without treatment). These results are in agreement with those reported by [13] who said that the elevation in plasma creatinine levels in dose of $AlCl_3$ -treated rats is considered as a significant marker of renal dysfunction. An evaluation of rodents' kidneys is the same as that for domestic animals, and also it involved increasing of urea nitrogen, creatinine. Present study revealed that AlCl3 administration caused increase in serum creatinine concentration. Statistically significant increase of serum urea and creatinine concentration in animals receiving Aliminum chloride is the consequence of critical accumulation of this metal in kidneys and following renal failure development and Aliminum is excreted mainly by kidneys [14].

Fig. 1 indicated that the level of ALT for G_1, G_2, G_3, G_4 and G_5 was 33.0, 39.0, 49.0, 57.0 and 63.0 U/L respectively. In analysis of variance, there is significant difference between groups G3, G4 and G5 at (P≤0.01), whereas there was non significant difference between groups G1 and G2. These findings indicated that the elevation of ALT level was associated with increasing the dose of treated Aliminum chloride in deionization water. Elevations in serum ALT activity are considered to be relatively specific for liver disease [15]. Therefore, the experimental rats particularly G3, G4 and G5 might be suffered from liver disease due to treatment of the deionization water with Aliminum chloride with high dose.

Fig. 2 indicated the level of AST for G_1, G_2, G_3, G_4 and G_5 was 37.0, 39.0, 45.0, 49.0 and 54.0 U/L respectively. In analysis of variance, there is significant difference between groups G_3, G_4 and G_5 at (P≤0.01), whereas there were non significant differences between groups G1 and G2. AST level for All experimental groups was within normal range of AST (7- 57 U/L). Elevated liver enzymes may indicate inflammation or damage to cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes, into the blood stream, which can result in elevated liver enzymes on blood tests. Elevated liver enzymes may be discovered during routine blood testing. In most cases, liver enzyme levels are only mildly and temporarily elevated. Most of the time, elevated liver enzymes don't signal a chronic, serious liver problem.

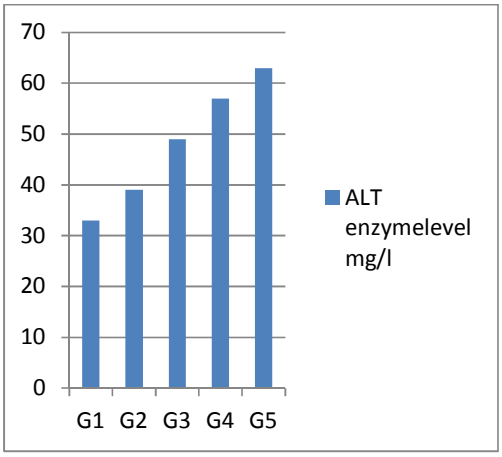


Fig. 1. ALT enzyme level of five experimental rat groups (U/L)

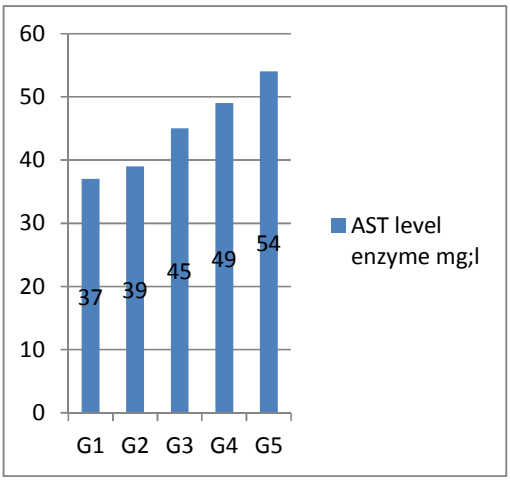


Fig. 2. AST enzyme level of five experimental rat groups (U/L)

3.1 Histopathology of Liver and Kidney of Rats that Treated and Untreated by Aluminum Chloride

Slide (1.1) indicated that the liver of rats in G1 (deionized water) was showed no lesions. While slide (1.2) indicated that the kidney of rats in G1 (deionized water) was showed dilatation and packing of the glomerular tubules. These results are in agreement with [16,17].

Slide (2.1) indicated that liver of rats in G2 (tap water) was showed no lesion. While slide (2.2) indicated that the kidney of rats in G2 (tap water) was showed the glomerular alteration, dilatation and shrinkage of the glomerular tubules [16,17].

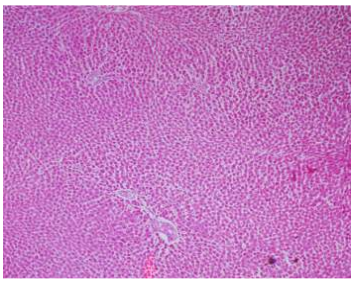
Table 1. Creatinine concentration for five groups

Experimental group	G1	G2	G3	G4	G5
Average±SD	0.5±0.02	0.62±0.09	0.70±0.00	0.96±0.14	1 .40±0.36

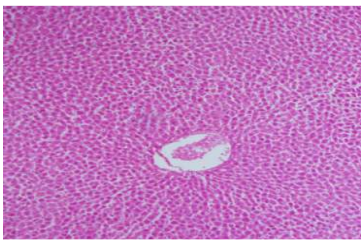
Slide (3.1) indicated that the liver of rats in G3 (50 mg \ kg Al CL_3) was showed cytoplasmic fatty change of the hepatocyte and isolated cell necrosis of the centrilobular hepatocyte. While slide (3.2) indicated that the kidney of rats in G3 (50 mg \ kg Al CL_3) was shown necrosis, fatty change, dilatation of renal tubules and segmentation of the glomerular tubules in cortex [16,17].

Slide (4.2) indicated that the liver of rats in G4(60 mg \ kg Al CL_3) was showed severe necrosis, while the kidney showed packing, dilatation of renal tubules and degeneration of glomeruli [16,17].

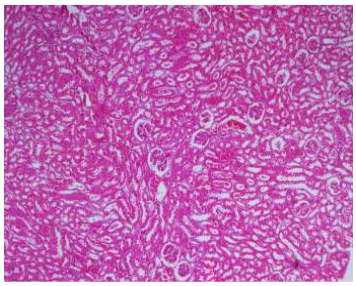
Slide (5.1) indicated that liver of rats in G5 (70 mg \ kg Al CL_3) was shown fatty cytoplasmic vaculation of the entralobular hepatocytes. While slide (5.2) indicated that kidney of rats in G5 (70 mg \ kg Al CL_3) was shown fatty change of the renal tubules in cortex. REF.



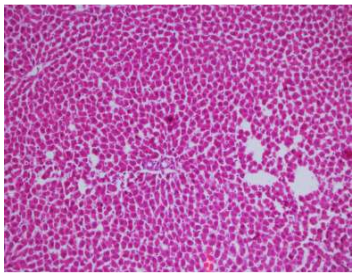
Slide 1.1. Shows liver of rats that received daily oral doses of deionized water (G1) for 8 weeks, no lesions were observed in liver. H&E x100



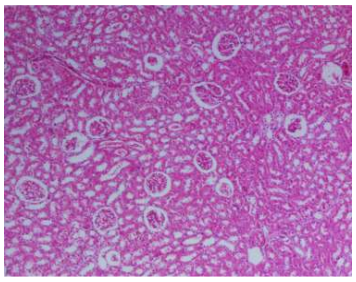
Slide 2.1. Shows liver of a rat that received daily oral doses of tap water (G2) for 8 weeks, no lesions were observed in liver. H&E x100



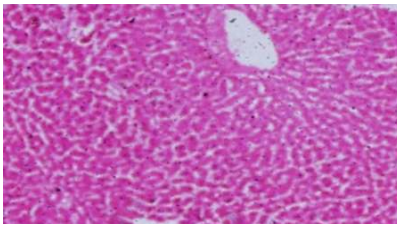
Slide 2.2. Shows Kidney of rat that received daily oral doses of tap water (G2) for 8 weeks, the glomeular alteration, dilatation, shrinkage of the glomeular tubules were observed in kidney H & E (X100)



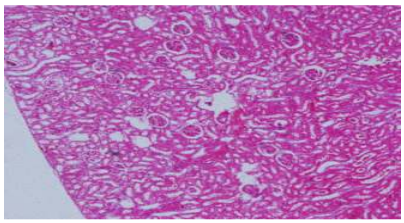
Slide 3.1. Shows liver of rats that received daily oral doses of 50 mg/kg Aluminum chloride (G3) for 8 weeks, the cytoplasmic fatty change of the hepatocyte and isolated cell necrosis of the centrilobular hepatocyte were observed in liver (H & E) ×100



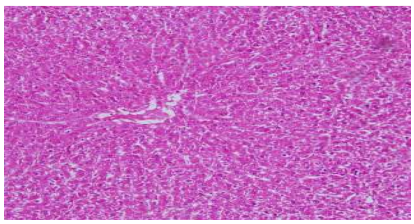
Slide 3.2. Shows Kidney of rat that received daily oral doses 50 mg/kg Aluminum chloride (G3) for 8 weeks, the necrosis, fatty change, dilatation of renal tubules and segmentation of the glomeular tubules in cortex were observed in kidney H & E (X100)



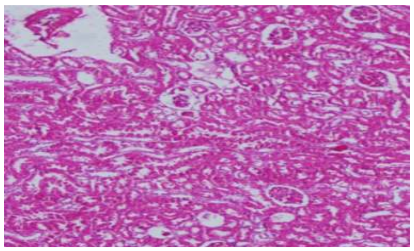
Slide 4.1. Shows liver of rats that received daily oral doses of 60 mg/kg Aluminum chloride (G4) for 8 weeks, no lesions were observed in liver (H & E) ×100



Slide 4.2. Shows kidney of rats that received daily oral doses of 60 mg/kg Aluminum chloride (G4) for 8 weeks, the severe necrosis, packing, dilatation of renal tubules and degeneration of glomeruli were observed in kidney H & E (X100)



Slide 5.1. Shows liver of rats that received daily oral doses of 70 mg/kg Aluminum chloride (G5) for 8 weeks. the fatty cytoplasmic vaculation of the entralobular hepatocytes were observed in liver H&E x100



Slide 5.2. Shows kidney of rat that received daily oral doses of 70 mg/kg Aluminum chloride (G5) 8 weeks, the fatty change of the renal tubules in cortex were observed in kidney. H&E x100

4. CONCLUSION

The present study indicated that oral administration of $AlCl_3$ at dose of 70 mg/kg daily for 60 days caused induced histological changes in the liver and kidney. There were increase in creatinine and liver enzymes (AST and ALT). Then Aliminum ion significantly increases creatinine and the activity levels of alanine aminotransferase, aspartate amino transferase. Consequently, there was cytogenetic injuries and damage to liver, kidney which induced by that molecules administration

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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