



# Evaluation and predictor ratio of toxicity of aluminium-tainted water impact in male rats: Oxidative stress in heart and kidney

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## ABSTRACT

Aluminium is a ubiquitous element; comprising about 8% of the earth's surface; and it has no known beneficial effect in humans, but can enhance adverse health effect. The adverse toxic effect of aluminium on body organs is incompletely understood, necessitating the need to better understand the mechanistic-link in its induced adverse health outcomes. This study evaluated the oxidative stress and established the predictor ratio of the negative impact of aluminium chloride - tainted water (AlCl<sub>3</sub>) assessed by alterations in pro-oxidant/antioxidant in the heart and kidney of male wistar rats. Fifty healthy male wistar rats were randomly assigned to five

groups of 10 rats each. Vehicle control group was given normal drinking water, while the treated animals were administered graded doses of  $\text{AlCl}_3$ , orally once daily for 28 consecutive days. Heart and kidney specimens were collected for assessment of oxidative stress markers-malondialdehyde (MDA) and protein carbonyl (PCO) and glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities. The results showed that  $\text{AlCl}_3$  in a dose-response-organ specificity induced significant higher oxidant/antioxidant enzymes' alterations in rats as reflected by marked increased MDA level with a concomitant decreased GPx, SOD and CAT activity. Correlation coefficient indicated that all the oxidative stress markers were significantly different upon comparing in both  $\text{AlCl}_3$  and control groups. In conclusion, our data indicated and confirmed that dose-response-organ dependent raised oxidative stresses are the possible mechanistic-link in  $\text{AlCl}_3$  induced male rat cardio-renal toxicity. Additionally, the established predictor ratio of the interplay in the interrelationship of the damaged tissue biomarkers can contribute to evaluation of pathophysiology risk.

**Keywords:** heart, kidney, oxidative stress, aluminium, predictor ratio, rats

## 1. INTRODUCTION

Aluminium so called the *soft-in-the-head* mineral is one of the most widely distributed element in the environment and the third most prevalence and bioavailability metal comprising 8% of the earth's crust ; and has not been identified as essential for any known biological function in man [1-3]. It is used in water purification and had found uses in industrial settings, and as pharmaceuticals, food additives, cosmetics and as other household products [3-5]. Moreover, there has been an increased incidence of exposure to the general population, in sub-Sahara Africa, Nigeria in particular; rain water collected from aluminium roofing constitutes the principal source for potable water demands, which can cause serious effect on various systems of the body including the cardiovascular-renal systems.

Cardiovascular diseases (CVDs) are the number 1 cause of death globally: more die annually from CVDs than from any other cause. WHO predicts 17.7 million deaths globally and over 75% deaths are in low-income and middle income countries; and are due to CVDs [6]. Kidney disease is a "hidden epidemic" accordingly, the overall prevalence of chronic kidney disease (CKD) in the general population is approximately 14%. More than 850 million people- 10% men and nearly 12% women around worldwide are affected. Almost half of individuals with CKD also have self reported cardiovascular disease (CVD), [7]. Indeed, exposure to aluminium has been implicated as a possible etiologic agent in a number of diseases [3, 8-13].

It has been suggested that the toxicity of aluminium depends on the form in which it occurs, while the mechanism of its action depend on the range of tolerance of an organism to aluminium concentration [14]. Besides, a body of evidence has accumulated implicating biochemical molecular mechanisms of aluminium toxicity to induced changes in biological antioxidant levels and production of excessive generation of free radicals, with subsequent oxidative stress [9, 15, 16].

Since information about data regarding the toxicity of aluminium in human and animal tissues are limited, the present study aimed to evaluate the oxidative stress as assessed by the alterations in antioxidant status in the heart and kidney in male wistar rats exposed to  $\text{AlCl}_3$  for 28 days. Predictor ratio evaluation of the interplay in the interrelationship between  $\text{AlCl}_3$  induced damaged tissue indices were also estimated.

## 2. MATERIALS AND METHODS

A committee of experts has reported that the oral median lethal dose (LD50) of aluminium chloride in rats ranges from 200 to 1000 mg of aluminium per kilogram of body weight [5]. The study protocol was approved by the Institutional Ethical Committee. Healthy fifty adult white male wistar rats weighing 100-120g were randomly divided into 5 groups of 10 rats each. Group 1 was given 2ml of water/day; whereas groups 2-5 were given 200, 400,600 and 800mg/kg/body weight per day of aluminium chloride –tainted water ( $\text{AlCl}_3$ ) respectively. The animals were housed in separate cages and fed with standard rat chow and water *ad libitum*.

### Bioassay of enzymatic and non-enzymatic antioxidants

The treatment period lasted for a total duration of 28 days. At the sampling time, the experimental animals were humanely scarified using diethyl ether anaesthetic agent and followed by cervical decapitation and the heart and kidney were quickly obtained and rinsed in ice cold physiological saline and prepared for tissue homogenate. The first-line defence enzymatic (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx) and non-enzymatic(malondialdehyde (MDA) and carbonyl protein (PCO)) antioxidants were estimated in the homogenate of the harvested heart and kidney crushed under ice for the control male wistar rats

and those administered various doses of aluminium chloride - tainted drinking water ( $\text{AlCl}_3$ ) according to the methods previously described [17-23].

### Statistical Analysis

SPSS version 20.0 was utilized to analyze the data. One Way Analysis of Variance (ANOVA) was used as the statistical tool and the results were presented as mean  $\pm$  SEM. Student's T-test was applied for further comparison, p-value < 0.05 marked for statistical significance.

### Conflict of Interest Statement

There are no conflicts of interest. This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## 3. RESULTS

### Effect of $\text{AlCl}_3$ on perturbations of antioxidant biomarkers in heart and kidney

The effects on some components of the antioxidant biomarkers -non-enzymatic oxidative systems in heart and kidney of male rats after 28 days of exposure to  $\text{AlCl}_3$  are compared in Tables 1&2. Tables 1&2 revealed that in rats of control groups and  $\text{AlCl}_3$  treated, the concentrations of MDA and PCO and activities of SOD, CAT and GPx in all studied tissues, were significantly affected by the types of organs and those treated with  $\text{AlCl}_3$  were markedly dose-dependent and types of organs. Our study showed that MDA level, an end product of lipid peroxidation, in the rats treated with  $\text{AlCl}_3$  was significantly increased ( $p < 0.05$ ) in a dose-response in the heart (+66.6%), (Table 1) and in the kidney (+245.8%), (Table 2) in 800mg/kg  $\text{AlCl}_3$  when compared to the vehicle control group. This gives a ratio of 1:4. MDA content was 11.8-fold higher in the kidney (55.9%) tissues compared to the heart (44.1%). Likewise, PCO an end product of protein also showed statistically significant increase in heart (+12.2%) and kidney (+37.7%) treated with 800mg/kg  $\text{AlCl}_3$  compared with control. This gives a ratio of 1:3.

In the enzymatic oxidative stress biomarkers, as presented also in Table 1 & 2,  $\text{AlCl}_3$  significantly induced decreases in a dose-dependent CAT, SOD, and GPx activities. The highest percentage of changes, after 28 days, in relation to the corresponding controls, for SOD, CAT and GPx activities in the kidney was -56.7%, -70.7%, and -84.4% (Table 2), respectively and in the heart -67.1%, -57% and -84.1% (Table 1), respectively in 800mg/kg  $\text{AlCl}_3$ . CAT, SOD, and GPx content of the heart was 59.3%, 37.2%, 49.1% compared to that of the kidney of 40.7%, 62.9% and 50.9% respectively. Predictor ratio revealed no significant difference in GPx activity in the kidney and heart.

Correlation coefficient analysis revealed that  $\text{AlCl}_3$  accumulated in the heart and kidney tissues and the levels of MDA and PCO exhibited a positive correlation, while negative correlation was found between MDA level and GPx, CAT and SOD activities. This indicated that the activities of SOD, CAT, and GPx decreased significantly with increasing the concentrations of Al accumulated in the heart and renal tissues, whereas the levels of MDA and PCO markedly increased.

**Table 1** Effect of  $\text{AlCl}_3$  induced changes in antioxidant levels in the heart of wistar rats

Dose mg/k body weight	MDA(ug/ml)	% change	PCO(g/l)	% change	Catalase (ug/ml)	% change	SOD(ug/ml)	% change	GPx(ug/ml)	% change
control	64.23 $\pm$ 0.78	0.0	11.04 $\pm$ 0.72	0	162.52 $\pm$ 10.16	0.0	26.99 $\pm$ 5.69	0.0	48.68 $\pm$ 1.59	0.0
200	77.76 $\pm$ 5.71 <sup>*,a</sup>	21.1	12.65 $\pm$ 0.46	14.6	103.50 $\pm$ 1.07 <sup>*,b</sup>	-36.3	9.39 $\pm$ 0.99 <sup>*,b</sup>	-65.2	36.76 $\pm$ 0.36 <sup>*,b</sup>	-24.5
400	79.35 $\pm$ 7.68 <sup>*,a</sup>	23.5	15.04 $\pm$ 1.17 <sup>*</sup>	36.2	133.79 $\pm$ 14.37 <sup>*,b</sup>	-17.7	18.28 $\pm$ 1.78 <sup>*,b</sup>	-32.3	24.75 $\pm$ 1.09 <sup>*,b</sup>	-49.2
600	89.14 $\pm$ 3.49 <sup>*,a</sup>	38.8	16.01 $\pm$ 1.56 <sup>*</sup>	45.0	84.41 $\pm$ 12.43 <sup>*,b</sup>	-48.1	14.93 $\pm$ 1.65 <sup>*,b</sup>	-44.7	16.66 $\pm$ 1.18 <sup>*,b</sup>	-65.8
800	107.00 $\pm$ 4.81 <sup>*,a</sup>	66.6	12.39 $\pm$ 0.31	12.2	53.43 $\pm$ 0.94 <sup>*,b</sup>	-67.1	11.61 $\pm$ 0.30 <sup>*,b</sup>	-57.0	7.74 $\pm$ 0.78 <sup>*,b</sup>	-84.1

Values are presented in mean  $\pm$  sem. N= 10. P  $\leq$  0.05 \*means values are statistically significant when compared to the control  
<sup>a</sup>=significantly higher between two biomarkers <sup>b</sup>= significantly lower between two biomarkers

### Predictor ratio of $\text{AlCl}_3$ induced perturbations of heart and kidney antioxidant damaged markers

Table 3 represented the predictor ratio analysis for  $\text{AlCl}_3$  induced alterations in antioxidant damaged markers in heart and kidney. The table showed that  $\text{AlCl}_3$  0, 200-600mg/kg induced alterations in the antioxidant in both the heart and kidney and ranked as: PCO:SOD:GPx:MDA:CAT; while at 800mg/kg  $\text{AlCl}_3$  was given as: GPx:SOD:PCO:CAT:MDA. Predictor ratio analysis revealed that in  $\text{AlCl}_3$  induced cardiotoxicity, PCO and MDA increased from the control values of 16.7% and 15.4% to 33.3% and 35.9% while GPx

and CAT decreased from 33.3% and 31.3% to 8.3% and 14.6% respectively in 800mg/kg AlCl<sub>3</sub>. SOD (28.6%) showed no significant difference across the groups. Whereas in AlCl<sub>3</sub> induced nephrotoxicity, predictor ratio revealed MDA and PCO increased from the control values of 11.1% and 16.7% to 46.7% and 33.3% respectively in 800mg/kg AlCl<sub>3</sub>. Concomitantly CAT, SOD and GPx decreased from the control values of 28.6%, 33.3% and 41.7% to 14.3%, 16.7% and 8.3% respectively in 800mg/kg AlCl<sub>3</sub>. The content of MDA, CAT, SOD and GPx in the heart as 45.3%, 53.9%, 38.7% and 53.4% and in the kidney as 54.8%, 46.4%, 62.9% and 46.6% respectively. Predictor ratio revealed that MDA and SOD content in the kidney was 9.5 and 24.2-fold high than that in the heart whereas CAT and GPx was 7.5 and 6.8-fold high in the heart compared to kidney ( $p < 0.05$ ), whilst the difference in PCO were not significant in heart and kidney ( $p > 0.05$ ).

**Table 2** Effect of AlCl<sub>3</sub> induced changes in antioxidant levels in the kidney of wistar rats

Dose mg/k body weight	MDA(ug/ml)	% change	PCO(g/l)	% change	Catalase (ug/ml)	% change	SOD(ug/ml)	% change	GPx(ug/ml)	% change
control	53.49 ± 2.08	0.0	11.14 ± 0.32	0.0	106.43 ± 1.01	0.0	45.48 ± 1.37	0.0	57.44 ± 0.44	0.0
200	73.37 ± 6.02 <sup>*,a</sup>	37.2	12.28 ± 1.23 <sup>*</sup>	10.2	84.20 ± 6.17 <sup>*,b</sup>	-20.9	34.46 ± 0.65 <sup>*,b</sup>	-24.2	39.58 ± 2.23 <sup>*,b</sup>	-31.1
400	93.18 ± 2.05 <sup>*,a</sup>	74.2	12.62 ± 0.27 <sup>*</sup>	13.3	69.50 ± 1.72 <sup>*,b</sup>	-34.7	28.54 ± 0.56 <sup>*,b</sup>	-37.2	27.75 ± 0.48 <sup>*,b</sup>	-51.7
600	95.77 ± 10.53 <sup>*,a</sup>	79.0	13.09 ± 0.14 <sup>*</sup>	17.5	57.10 ± 2.73 <sup>*,b</sup>	-46.3	14.29 ± 1.55 <sup>*,b</sup>	-68.6	12.80 ± 0.64 <sup>*,b</sup>	-77.7
800	184.9 ± 61.30 <sup>*,a</sup>	245.8	15.34 ± 1.32 <sup>*</sup>	37.7	46.10 ± 0.40 <sup>*,b</sup>	-56.7	13.31 ± 0.60 <sup>*,b</sup>	-70.7	8.97 ± 0.32 <sup>*,b</sup>	-84.4

Values are presented in mean ± sem. n = 10.  $P \leq 0.05$  \*means values are statistically significant when compared to the control; <sup>a</sup>=significantly higher between two biomarkers; <sup>b</sup>= significantly lower between two biomarkers

**Table 3** Predictor ratio of association of AlCl<sub>3</sub> induced damaged antioxidant indices in heart and kidney

Dose/mg/kg /body weight	Ratio of association of antioxidant indices in kidney	Ratio of association of antioxidant indices in heart
Control	PCO[1]:SOD[4]:GPx[5]:MDA[5]:CAT[10]	PCO[1]:SOD[2]:GPx[4]:MDA[6]:CAT[15]
200	PCO[1]:SOD[3]:GPx[3]:MDA[6]:CAT[7]	PCO[1]:SOD[1]:GPx[4]:MDA[8]:CAT[11]
400	PCO[1]:SOD[2]:GPx[2]:MDA[7]:CAT[6]	PCO[1]:SOD[1]:GPx[2]:MDA[5]:CAT[9]
600	PCO[1]:SOD[1]:GPx[1]:MDA[6]:CAT[6]	PCO[1]:SOD[1]:GPx[1]:MDA[6]:CAT[6]
800	GPx[1]:SOD[2]:PCO[2]:CAT[5]:MDA[21]	GPx[1]:SOD[2]:PCO[2]:CAT[7]:MDA[14]

#### 4. DISCUSSION

In the present study, we had examined and evaluated the implication of antioxidants as predictors of risk of developing cardiovascular-renal disorders among male wistar rats exposed to aluminum-tainted water for 28 days. Additionally, predictor ratio that explored the interplay of interrelationship in AlCl<sub>3</sub> induced perturbations in oxidative stress damaged markers has been established. The generalized concept is that AlCl<sub>3</sub> inactivation of antioxidant enzymes leads to lipid peroxidation and oxidative damage in cells and tissues. Accordingly, aluminium can induce nephrotoxicity [15,24,25] and cardiotoxicity [11,16], mainly by its deleterious effects on lipid peroxidation which is the main manifestation of oxidative damage. A striking observation was that AlCl<sub>3</sub> accumulated differentially in the heart and kidney tissues; and in a dose-response-organ-dependent enhanced acted as predisposing factor for oxidative stress by producing significant toxic potential which altered pro-oxidants and antioxidants homeostasis mechanisms. This is an indication that not all routes of exposure are equivalent in their delivery of aluminium to target sites. Significantly, the differential imbalances were evidenced by AlCl<sub>3</sub> deregulated CAT, SOD and GPX enzymatic activities that constituted a mutually primary cellular defense team against free radical mediated oxidative stress in the heart and kidney. Using the predictor ratio analysis, the overall data of CAT and GPX enzymes activity calculated for the cardiac tissue was 7.5-fold and 6.8-fold respectively higher than that found in the renal tissues; whereas SOD was 24.2-fold higher in the renal tissues than in cardiac tissues when compared to the control. Our results are in basic agreement with the previous findings [27] which reported that CAT enzyme activity showed significant decrease in renal tissues compared to heart tissues. As was observed, an increased SOD enzyme activity (62.9%) was thought to be secondary to decreased CAT activity (40.7%) in the kidney; and on the other hand, an increased CAT activity (59.3%) was secondary to decreased SOD activity (37.2) in the heart tissue, as compensation mechanisms, preventing the kidney and heart tissues respectively from destruction induced by aluminium. Besides, MDA content, on the other hand, a non-enzymatic antioxidant and an end product of lipid peroxidation, in the rat treated with AlCl<sub>3</sub> was significantly increased 9.5-11.8-fold

higher in the kidney tissues compared to the heart. These are suggestive of mechanistic-link between risk of kidney and cardiovascular diseases. This may provide evidence that pathophysiological derangements implicating kidney disease and toxin accumulation could contribute directly to progression of cardiovascular disease and adverse outcomes [28].

Biochemically, as observed elsewhere (and in our unpublished work), aluminium accumulated in the kidney resulted in disruption in biochemical homeostasis accompanied with statistically significant increase in renal markers (serum urea and creatinine), is an indication of renal dysfunction [15, 16, 24].

As observed in the present study, predictor ratio revealed that there was no difference in the content of the marker of free radical processes in vivo, PCO and cellular antioxidant defense marker GPx in both kidney and heart tissues. AlCl<sub>3</sub> significantly inhibited GPx activity in the heart and kidney.

## 5. CONCLUSION

In our study observed, all the oxidative stress indices were found to be dose-response-organ specific enhanced significantly different upon comparing these indices in both AlCl<sub>3</sub> and control groups. Elevated levels of oxidant/antioxidant enzymes' alterations in AlCl<sub>3</sub>-induced cardiac-renal tissues and cells damaged contributed significantly to the mechanistic-link of AlCl<sub>3</sub> induced cardio-renal risk in rats and which requires therapeutic approach to these events. Furthermore, the established predictor ratio of the interplay in the interrelationship in AlCl<sub>3</sub> induced damaged tissue biomarkers could be beneficial as sensitive index for estimates of risks.

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