Biochemical Effects Of Aluminum On Some Selected Serum Enzymes Of Male Wistar Albino Rats

Ogueche, P.N,Ogbu, A.E, Dike, C.C, Ugwu, C.E, Okonkwo, C.O.

Abstract: Toxic metals are widely found in our environment and humans are exposed to them via water, contaminated air, food and soil. Aluminum (AL) belongs to this group of toxic metals. Its neurological effects are well documented but effects on acid and alkaline phosphatases are poorly studied and this the essence of this study. Toxicity of aluminum was investigated based on the elevation of acid and alkali phosphatases in serum of male Wistar albino rats after days 7 and 14 of aluminum (0.38, 3.8 and 38mg/kg body weight) administration respectively. The results showed significant increase ($p<0.05$) in serum acid phosphatase in the test animals given 38kg/kg after days 14 while serum alkali phosphatase increased significantly ($p < 0.05$) in the test animals given 3.8 and 38 mg/kg after days 7 and 14 when compared to the control animals. However, lower dose (0.38mg/kg) showed increase in both serum acid and alkali phosphatases respectively but were statistically non-significant ($p>0.05$) at 7 and 14, as compared to control animals.

Index Terms: Aluminum, Acid phosphatase and Alkali phostphatase, Serum, Toxicity.

1 INTRODUCTION:

Aluminum, (Al) is the third most abundant element in the earth's crust. It is chemically reactive metal and does not occur naturally in its elemental form, but is found in combination of oxygen, fluorine, silicon, and other elements in the soil, rocks, clays, etc [1].It is absorbed by many plants and occurs in plant products in the diet. The daily ingestion of Al by humans is estimated to be 30 to 50 mg [2] however, the use of Al-containing antacids may increase daily ingestion of Al doses from 50–1000 mg/day [3]. In addition, to the leaching of Al from the soils by acid rain which is increasing free Al in the environment and in the surface waters, the general population is also exposed to Al from its widespread use in water treatment, as a food additive and colorants, drying agents from various Al based pharmaceuticals, from occupational dusts, and from Al cans and containers as well as cooking utensils [4] The populace is also exposed to Al through the use of deodorants, packaging foil, lip-sticks, and tooth pastes, drugs (vaccines, antacids, and phosphate binders) as well as drying agents and water flocculent in water plants [5,6,7] Perhaps the numerous applications of Al is because of its light-weight, corrosion-free, and relative inexpensiveness [8].

- *Ogueche, Peter Nnamdi is currently a lecturer in the Department of Human Biochemistry Nnamdi Azikiwe University,Awka, Nnewi Campus,Anambra State Nigeria. Email: pn.ogueche@unizik.edu.ng Postal code: 435001*
- *Ogbu, A.E.; is currently a Ph.D student in the Department of Biochemistry University of Nigeria, Nsukka. Enugu State, Nigeria*
- *Dike, C.C is currently a senior Laboratory Scientist in the Department of Human Biochemistry Nnamdi Azikiwe University,Awka, Nnewi Campus,Anambra State Nigeria*
- *Ugwu,C.E is currently a lecturer in the Department of Human Biochemistry Nnamdi Azikiwe University,Awka, Nnewi Campus,Anambra State Nigeria*
- *Okonkwo, C.O. is currently a lecturer in the Department of Human Physiology, Nnamdi Azikiwe University,Awka, Nnewi Campus,Anambra State Nigeria*

However, Al is not essential for organisms and no biological function has been assigned to it [9] however, its accumulation in tissues and organs has been reported to result in their dysfunction and toxicity [10]. Al compounds are being used in many industrial as well as house hold applications like water treatment, drugs and utensils, etc [11] .Al transverses across the cell membrane and enters into the blood circulation where it binds to the serum proteins, particularly transferrin [12]. This mechanism is receptor-mediated endocytosis, via aluminum transferrin complex. Al is then absorbed by cells through transferrin receptor similar to iron absorption [13]. Al has potential to be toxic to humans. The Agency for Toxic substances Diseases Registry [14] reported that Al is distributed mainly in the bone, live, testes, kidneys and brain. The human toxicological effects include encephalopathy, bone disease, anemia and skeletal system disease and aluminum is a possible contributing factor in diseases such as Alzheimer's, Parkinsonism, Dementia and Amyotrophic lateral sclerosis [15,16,17]. However, despite the known neurotoxicity of Al ,biochemical effects of Al in serum acid and alkali phosphatases is poorly studied and this is the purpose of the study The objective of the study is to investigate the biochemical effects of Al administration in rats as animal model using serum acid and alkali phosphatases as markers of toxicity.

2 MATERIALS AND METHODS

2.1 Materials:

Twenty-four (24) male wistar albino rates aged between 8-10 weeks with a body weight range of 150-205g were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The Institutional Animal Ethics Committee approved the study before the experiment and certified all experimental protocols/parameters The toxicant administered daily to the experimental animals was aluminum as in aluminum chloride ($AlCl_3$) at varying doses: 0.38, 3.8 and 38mg/kg body weight while the control animals received normal saline (0.2ml). All chemicals used were of the analytical grade.

2.2 Methods:

The animals were housed and distributed randomly in four separate metabolic cages of six each differentially marked and acclimatized for five days. The four groups were labeled A-D. while group A is the control administered 0.2ml normal saline used in dissolving the toxicant, groups B, C and D were the groups administered 0.38, 3.8 and 38mg/kg body weight aluminum as aluminum chloride ($AlCl_3$) daily respectively. The route of administration was oral by means of gastric intubations. All animals were fed with commercial feed (grower's mash) and water *ad libitum* for fourteen days. Each experiment was in triplicate and results were pooled. Blood was collected from each group after seven and fourteen days through the median cantus vein in the eyes of the rats with the aid of capillary tubes and transferred into plastic tubes. This was later centrifuged at 2000 xg and serum collected into separate test tubes. The sera were used for analysis.

Acid and Alkali Phosphatase

Acid and alkali phosphatase in sera samples were assayed as the condensation of amino-antipyrine and phenol using the methods described by King and Kind, 1952 [18]. Oxidation of the condensation product with ferricyanide gives a red colour whose intensity at 520nm is proportional to acid and alkali phosphatase activities respectively. Note that the two parameters have similar principle, while disodium phenyl phosphate (substrate) is buffered with citric acid sodium buffer for acid phosphatase the alkali phosphatase is buffered with alkali sodium carbonate buffer. In this method, duplicate samples of four test tubes labeled thus: T-test, C-control, S- standard and B- blank were used. Into tubes T and C were pipetted 1.0ml each of citric acid buffer (for acid phosphatase) and carbonate buffer (for alkali phosphatase) and disodium phenylphospate solution respectively. The solutions were mixed and warmed for three minutes at 37^0C. To tube T was added 0.Iml of serum, it was mixed and incubated for 15minutes at 37^0C. To tubes S and B were pipetted 1ml of citric acid buffer for acid phosphatase and 1ml of carbonate buffer for alkali phosphatase as well as distilled water (1ml), and working phenol standard respectively. Sodium hydroxide (0.5N)(0.5ml) was added to all four tubes. Sodium bicarbonate solution (1ml), 4- amino-antiprine solution (1ml) and potassium ferricyanide solution (1m) were added to all four tubes sequentially with mixing after each addition. The absorbance of the contents of the tubes, T, C and S were read against blank at 520nm. Acid and alkali phosphatase activities in king-Armstrong unit per 100ml (K-A. U/100ml) was calculate thus.

$$\frac{A(T) - A(C) \times 10\ (I\ U)}{A(S)}$$

Where A(T) = Absorbance of test Sample
A(C) = Absorbance of control
A(S) = Absorbance of standard

2.3 Statistical analysis:

Significant differences were assessed by one-way analysis of variance (ANOVA) while differences between treatment animals were calculated using student's independent t-test. The acceptance level of significance was ($P < 0.05$) using a two-tail distribution.

3.0 RESULTS.

Effects of Aluminum on some selected serum Enzymes (Acid and Alkali Phosphatase Activities).

3.1 Acid phosphatase Activity:

our results in table 1.0 below show that at seven days of Al administration, the acid phosphatase level increased in all the test animals but were statistically non-significance ($p>0.05$) as compared to the control animals. In the contrary, after fourteen days of Al administration, the acid phosphatase (AP) activity of the test animals given 38mg/kg body weight significantly increased ($p< 0.05$) as compared to the control animals. Similarly, the test animals given 0.38 and 3. 8mg/kg body weight showed increase in acid phosphatase activity but were not statistically significant ($p > 0.05$) relative to the control. The test animals given 0.38mg/kg had the least activity of acid phosphatase relative to other test animals. The acid phosphatase activity was significantly higher ($p< 0.05$) within the test animals given 0.38 and 38mglkg body weight. This is unlike test animals given 3.8 and 38mglkg body weight of Al. This is shown in the table below.

3.2 Alkali Phosphatase Activity:

As seen in tables I and 2 below, the alkali phosphatase (ALP) activity were significantly higher ($p< 0.05$) in the test animals given 3.8 and 38mg/kg body weight after days 7 and 14 respectively as compared to the control animals. The test animals given 0.38mg/kg after day 14 of Al intoxication had the least value of alkali phosphatase activity as compared to the control and other test animals but were not statistically significant ($p > 0.05$). Within the test animals (0.3mglkg and 3.8mglkg) and (0.38 and 38 mg/kg) body weight showed significant difference ($p < 0.05$) in alkali phosphatase activity after fourteen days of Al administration.

4 DISCUSSION.

In toxicological studies, investigating the effects of toxic elements/chemicals at environmentally relevant concentration is always sought. The concentrations of aluminum as in aluminum chloride used in the present study are within the range of human exposures. Nevertheless, searching for the proper elucidation of the molecular basis of Al toxicity has stimulated numerous experimental studies. Yet the actual molecular mechanism of Al toxicity is not well understood. In the present study, efforts were made to investigate the biochemical effects of Al on some selected serum enzymes: acid and alkali phosphatases using rats as experimental animals. The results showed that Al administration in rats elevated these serum enzymes after days 7 and 14. Acid phosphatase is an enzyme abundant in the prostate and seminal fluid. It also occurs in significant amounts in many other tissues such as spleen, liver, kidney, red cells and bone [19]. Increased production of the enzyme-acid phosphatase observed with increased exposure to Al suggests that exposure to Al increases the release of the enzyme from the prostate into the blood stream and thus may enhance the risk of prostate problems. Similarly, our study revealed

increase in alkali phosphatase (AP) activity in the test animals given 3.8 and 38mg/kg $AlCl_3$ after fourteen days of exposure as compared to the control animals. Alkaline phosphatase (ALP) is a marker enzyme for the integrity of the hepatobiliary system and the flow of bile into the small intestine. The increase in serum ALP activity indicates obstructive event or cholestatic effect following Al exposure. Data from this study indicate that Al administration leads to elevation in serum enzymes: acid and alkali phosphatases, which was dose and duration dependent. In another study reported elsewhere [20] stated that Al accumulation in tissues of experimental animals were dose and duration dependent. According to the Canadian Association of Gastroenterology practice guidelines [21], an elevation in alkali phosphatase (AP) activity is linked with cholestatic disease, pregnancy, bone disease and occasionally with inflammatory bowel disease. However, alkali phosphatase elevation does not always signal a disease state,]22a]. Growing bones need AP and any condition of bone growth will cause an increase in alkali phosphatase production. The condition may be normal such as in childhood growth sport or healing of a bone fracture or disease state, such as bone cancer, Paget's disease, or rickets. [22b] also reported that during pregnancy, AP made by the placenta leaks out into the mother's bloodstream. Data from this study show that exposure to Al increased the level of AP in the serum. This suggests that Al intoxication can predispose animals to the risk of diseases associated with high alkali phosphatase levels. In another study [24] reported that Al administration to rats leads to serum elevation of aspartate transaminase (AST) activity, suggesting possible liver damage.

5. CONCLUSION.

This work suggests that Al administration to male Wistar albino rats produced biochemical effects evidenced in elevation of serum enzymes such as acid and alkali phosphatases which may predispose the test animals to cholestatic disease as well prostate problems.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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Table 1: *Effect of Aluminum on serum Acid and Alkali phosphatase Activities of Control and Test Animals after Seven (7) days of Al Administration in Rats*

Serum Enzyme (IU)	Control	0.38mg/kg	3.8mg/kg	38mg/kg
Acid phosphatase	23.93+2.96	25.57+ 4.31	28.27+1.92	30.27±1.72
Alkali Phosphatase	51.57+ 9.50	50.60+7.60	50.20+8.76*	60.30±4.88*

Table 2: *Effect of Aluminum on serum Acid and Alkali phosphatase Activities of Control and Test Animals after Fourteen (14) days of Al Administration in Rats*

Serum Enzyme (IU)	Control	0.38mg/kg	3.8mg/kg	38mg/kg
Acid phosphatase	26.23+4.00	^{a}28.57+ 2.02	34.60+4.03	^{b}38.00+2.20*
Alkali Phosphatase	51.63+ 8.54	^{a}49.70 + 6.12	^{b}57.20+5.45*	^{c}63.90+5.50*

n = 6

Means + SEM, Means with asterisk in a row are statistically significant ($p < 0.05$) between control and test animals while different superscripts in a row are statistically significant ($p < 0.05$) within the test animals.