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Original Research Article

Effects of *Moringa oleifera* Seeds on the Serum Electrolytes of Wistar Rats Intoxicated with Aluminum Chloride

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ABSTRACT

The concentration of serum electrolytes is one of the most commonly used laboratory tests for assessment of a patient's clinical conditions and disease states, this is because electrolyte balance is essential for normal functioning of cells and organs of the body. The current study examined the effects of aluminum chloride ($AlCl_3$) on the serum electrolytes, liver enzymes of 35 wistar rats divided into seven groups of five (n = 5) and treated with 100 and 400 mg/kg body weight of the ethanol and aqueous seed extracts of *Moringa oleifera*. The study showed the lowering effects of *Moringa oleifera* on serum electrolytes levels. Further studies showed that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly ($p<0.05$) increased from 11.91 ± 0.20 and 11.83 ± 0.40 to 59.23 ± 0.24 and 57.29 ± 0.50 respectively when rats were induced with aluminum chloride toxicity. Upon treatment with ethanol and aqueous extracts of *Moringa oleifera* at 100 mg/kg, the levels of ALT and AST significantly ($p<0.05$) dropped. An increase in the concentrations of the extracts to 400 mg/kg resulted to a significant ($P<0.05$) elevation of ALT 11.93 ± 0.17 (ethanol) and 11.94 ± 0.07 (aqueous) and AST 11.77 ± 0.25 (ethanol) and 11.78 ± 0.15 (aqueous). The results of this study show that at lower concentrations *Moringa oleifera* seeds are effective against $AlCl_3$ by regulating levels of serum electrolytes, ALT and AST in wistar rats.

Keywords: Electrolytes, *Moringa oleifera*, Aluminium Chloride, Seed extracts.

Introduction

Plants products and their derivatives have been considered as an origin of therapeutic elements from ancient times.[1] Today, there is basic research motivation in essential oils and extracts from different plant sources as potential antioxidant materials. *Moringa oleifera* is known to have impressive range of medicinal uses and high nutritional value. Nearly every part of this plant, including its root, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil have been used for treatment of various ailments in indigenous medicine.[2-3] Studies on animal and human models have demonstrated these benefits attributed to it. For instance, *Moringa* flowers are used as traditional remedies for tumours, the seeds and leaves are applied as poultice to sores and rubbed on the temples of the head to relieve pains and headaches.[4]
One of the most common elements in the earth's crust is Aluminum (Al), a large amount of it is distributed in the environment and people use it extensively in their daily life. Aluminum can get into the body either from the environment or from diet.[5] As a result of its extensive use in daily life, humans are easily exposed to aluminum. The compounds can be used in pharmaceuticals, in water treatment process, in food additives and also in consumer products. Dietary aluminum is mostly gotten from corn, salt, herbs, spices, tea, cosmetics, aluminum wares and containers. Environmental pollution exposes people to levels of this compound above the normal range. The particulate matters released by cement-manufacturing plants contain high amount of Aluminum, this makes people residing within such environment at higher risk.[6]
Medications such as antacids, buffered aspirin, anti-diarrheal products, vaccine and allergen injection have aluminum incorporated in them. It is used as a component of veterinary medicine, glues and disinfectants. In the purification process of drinking water, aluminum sulphate is extensively added as a coagulant. Due to its serious effects on the central nervous system,[7] metabolism and hematology, fertility and reproduction,[8] embryotoxicity and teratogenic potency, aluminum and its compounds are receiving an increased level of attention.
Electrolytes are ions that regulate the electric charges on cells and the flow of water across their membranes. The most common electrolytes regulated in the body are sodium, potassium, and chloride.[9] Because a balance of electrolytes in the body is vital for optimum functioning of cells and organs; serum electrolytes concentration is one of the common laboratory tests used to assess a patient's clinical conditions and disease states.[3]
Sodium (Na^+) is the most abundant cation in the extracellular fluid and the major factor that regulates the body water balance. The major function of sodium is to regulate serum osmolality and fluid balance[10] but it is important in maintaining the transmembrane electric potential for action potential and neuromuscular functions.
The regulation of water and acid-base balance in blood and tissue is controlled by potassium along with sodium.[11] Potassium is important in the regulation of heart beat and function of muscles. In mammals, electrolytes like sodium and potassium are primary in the maintenance of osmotic pressure and water distribution; they also have roles in maintaining pH, oxidation reduction reactions, heart muscle functioning and as cofactors for enzymes.[12]
Chloride is the most abundant extracellular anion. In the renal proximal tubules, where it is primarily regulated, chloride is exchanged for bicarbonate ions and passively follows sodium and water throughout the rest of the nephron. Cl^- is influenced by the extracellular fluid balance and acid-base balance.[13] Homeostatic mechanisms indirectly regulate Cl^- through changes in sodium and

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bicarbonate. The physiological role of chloride is to balance out positive charges in the extracellular fluid and, by following sodium passively, it helps to maintain extracellular osmolality.

Materials and Methods

Sample collection and preparation

Dried seeds of *Moringa oleifera* were collected from a healthy plant from its natural habitat around the Wukari area of Taraba State on Friday 26th June, 2020 and was sent to the International Centre for Ethnomedicine and Drug Development in Nsukka, Enugu State where it was identified and authenticated by Ugwu Paschal Ifeanyichukwu (Herbarium Curator) and Alfred Ozioko (taxonomist) as *Moringa oleifera* Lam: Voucher number: CEDD/025. The dried seeds of *Moringa oleifera* were pulverized to powdered specimen using a mortar and pestle.

Sample extraction

The powdered seeds were weighed and 200 g was macerated in ethanol and another 200 g was macerated in water (for the aqueous) in the ratio 1:5 for exactly 48hrs. The extracts were filtered first using a clean white sieving mesh and then using the Watman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator at reduced pressure. The concentrated extracts were then transferred to air-tight containers, preserved in the refrigerator at 4 ℃ until required.

Animal specimen

Wistar rats (35) weighing 100–150 g were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in clean cages and maintained under standard laboratory conditions (Temperature 25 ± 5 ^{o}C, Relative humidity 50 – 60 %, and a 12/12h light/dark cycle) and allowed free access to standard diet and water ad libitum. Animals were allowed to acclimatized for 7 days before each of the experiments. All experimental procedures were approved to be in compliance with the ethical guide of the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (IACUC, FVM UNN) with the Approval Reference Number: FVM-UNN-IACUC-2021-0569.

Experimental design

The rats were randomly divided into seven groups of five animals each (n = 5). Group 1 (the control group) received normal feed and water + 3 % tween 80 after 1 hour. Group 2 received 100 mg/kg bw Aluminium chloride + 3 % tween 80 after 1 hour. Group 3 received 100 mg/kg bw standard drug an hour after administering 100 mg/kg bw of Aluminium chloride. Group 4 received 100 mg/kg bw ethanol extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride. Group 5 received 100 mg/kg bw aqueous extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride. Group 6 received 400 mg/kg bw ethanol extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride. Group 7 received 400 mg/kg bw aqueous extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.

After the experimental period, animals were sacrificed and venous blood was collected by ocular puncture. Blood samples were collected into plain sample tubes containing no anticoagulant for the serum. The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 min.[14]

Biochemical assay

Estimation of the potassium

Potassium (K^+) was estimated according to method of Tietz *et al.* (1976).[15]The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7 mEq/L.

Estimation of the Bicarbonate

Bicarbonate was estimated according to the method of Tietz *et al.* (1976).[15]

Phosphoenol Pyruvate carboxylase (PEPC) catalyzes the reaction between Phosphoenol Pyruvate and carbon dioxide (bicarbonate) to form oxidation and Phosphoenol ion. Oxalacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD, the reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340 nm that is directly proportional to CO_2 concentration in the sample.

Estimation of the chloride

Chloride was estimated according to the method of Tietz *et al.* (1976).[15]

Chloride ions form a soluble, non-ionzed compound, with mercuric ion and will displace thiocyanate. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 480 nm. The intensity of the color produced is directly proportional to the chloride concentration.

Determination of serum magnesium concentration

The sample (0.1 ml) was taken in triplicate, 0.9 ml of distilled water, 2 ml of 10% sodium tungstate, 2 ml of 0.67 N H_2SO_4 was added and centrifuged at 3000 rpm for 10 min. The filtrate was collected and 1 ml of distilled water, 1 ml of 0.1% acacia and 1 ml of 0.05% titan yellow was added and shaken for 5 min. Then, 2 ml of 4 N NaOH was added and the absorbance measured at 520 nm.

Determination of serum calcium concentration

Triplicate of the sample (2 ml) was pipette in a tube, few drops of saturated ammonium oxalate solution were added and the resultant precipitate of calcium oxalate was dissolved in H_2SO_4. The Oxalic acid formed was titrated with a standard solution of $KMnO_4$, the quantity of calcium in the serum was calculated as 1 ml. N KMnO4 = 2.005 mg Ca.

Determination of serum sodium

1.0 ml filtrate reagent was added both in blank, standard and sample test tubes and 50 µl of the distilled water, standard reagent and sample was added. Then, shake all tubes vigorously and mix continuously for 3 minutes and centrifuge at high speed (1,500G) for 10 minutes and test the supernatant fluids. After that, pipette 1.0 ml of the acid reagent to all test tubes. Add 50 µl of the supernatant to respective tubes mix. Again, add 50 µl of the color reagent to all tubes and mix. Measure the absorbance measured at 550 nm.

Determination of the aspartate aminotransferase (AST) activity

The activity of aspartate aminotransferase was assayed by the method of Reitman and Frankel (1957)[16] as outlined in the Randox kit used. Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazine with 2,4-dinitrophenylhydrazine.

Determination of the alanine aminotransferase (ALT) activity

The activities of alanine aminotransferasewas assayed by the method of Reitman and Frankel (1957)[14] as outlined in Randox Kit.

Determination of the alkaline phosphatase (ALP) activity

The activities of alkaline phosphatase were assayed by the method of Babson *et al.* (1966),[17] as outlined in Randox kit, used. The alkaline phosphatase act upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) using Graph Pad Prism version 5 and Tukey post hoc test. Values of $P < 0.05$ were considered to be significant. The data obtained were expressed in tables and charts.

Results and Discussion

Table 1 shows the result of serum electrolyte activity in rats treated with $AlCl_3$ singly and also ethanol and aqueous extracts of *M. oleifera*. The present result showed significant decrease of potassium K, malcium Ca, magnesium Mg, and sodium Na as compared with the normal control, except for chloride Cl, and bicarbonate Bi-C, which were significantly increased as compared with the normal control in Group 2 animals (treated with $AlCl_3$ only).

When compared to the control, group 1, potassium levels decreased in groups 4 to 7 from 4.37 ± 0.33 to 4.09 ± 0.80, 4.02 ± 0.43, 4.30 ± 0.17 and 4.30 ± 0.36 respectively. Calcium was also observed to have decreased in a similar manner from 9.81 ± 0.50 in group 1 to 9.13 ± 0.74, 8.95 ± 0.80, 9.70 ± 0.89 and 9.47 ± 1.19 in group 4 to 7 respectively. Magnesium, Mg concentrations also decreased noticeably from 2.19 ± 0.35 to 2.09 ± 0.31, 1.96 ± 0.20, 2.27 ± 0.26 and 2.25 ± 0.15 in group 4 to 7. Sodium Na, Chloride Cl, and bicarbonate also decreased in these same groups.

Effects of $AlCl_3$ on serum electrolytes

Electrolytes are substances that become ions in solution and acquire the capacity to conduct electricity; sodium, potassium, and chloride are among the most commonly monitored electrolytes in the body. Serum electrolyte concentration is among the most commonly used laboratory tests for assessment of a patient's clinical conditions and disease states, because electrolyte balance in the body is essential for normal functioning of cells & organs.[18]

The body system has been fashioned in a manner that it can tackle invading foreign substances in most cases, the body system needs to be protected, enhanced and activated.[19] The ability to activate the body defense mechanism or to protect the body system has been found to be present in some plants such as *M. oleifera*. *Moringa oleifera* has been a useful plant in the treatment of so many diseases but there seem to exist very little research on this plant in the area of serum electrolytes.

In the current studies, results show that $AlCl_3$ has lowering effects on serum electrolytes levels (Table 1). Levels of K^+ decreased from 4.37 ± 0.33 in the control (group 1) to 3.36 ± 0.13. Upon treatment with 100 mg/kg bw of the ethanol and aqueous extracts of *M. oleifera* seeds the K^+ increased to 4.09 ± 0.80 and 4.02 ± 0.43 respectively. *M. oleifera* is known to have blood pressure lowering effect[20] but it was still able to increase the levels of serum K^+. When the concentration of the extracts was increased to 400 mg/kg bw, K^+ levels further elevated to 4.30 ± 0.17 and 4.30 ± 0.36. This suggest that higher doses of *M. oleifera* seeds extracts will increase K^+ levels.The concentration of Ca also decreased from 9.81 ± 0.50 to 7.58 ± 1.83 after administration of 100 mg/kg bw of $AlCl_3$ when treated with 100 mg/kg bw of the ethanol and aqueous seed extracts *M. oleifera* the Ca levels improved significantly to 9.13 ± 0.74 and 8.95 ± 0.80 respectively. Increasing the dosage to 400 mg/kg bw yielded in increase to 9.70 ± 0.89 and 9.47 ± 1.19 respectively.

Similar patterns of increasing concentrations were noticed in both Mg and Na^+ levels except for the levels of Na^+ that was slightly lower in the group treated with 100 mg/kg bw of aqueous and ethanol extracts of *M. oleifera* seeds as compared to groups treated with higher doses of 400 mg/kg bw of aqueous and ethanol extracts of *M. oleifera* seeds. Cl^- and bicarbonate levels went in an opposite manner to the others was decreased by action of $AlCl_3$, but when treated with 100 mg/kg ethanol and aqueous extracts of *M. oleifera* seeds the levels were significantly lowered. This is mostly because of the blood lowering capability of *M. oleifera*. Amazingly increasing the concentrations of the seed extract to elevated the levels of chloride and bicarbonate. This suggest that higher levels of the seeds extracts increases serum chloride and bicarbonate levels. It has been seen that even synthetic drugs have side effects and this may possibly be the side effects of *M. oleifera* seeds extracts because elevation patterns were observed in both extracts.

Presented in table 2 is the result of serum liver enzymes of rats treated with $AlCl_3$, ethanol extracts and aqueous extracts of *M. oleifera*. The result showed significant elevation in the concentrations AST, ALT and ALP levels in the group treated with $AlCl_3$. The result further revealed that admiration of $AlCl_3$ along with the extracts of *M. oleifera* seeds reduced such concentrations.

Effects of $AlCl_3$ on Liver Enzymes

Vital in various metabolic processes are the liver and kidneys, this exposes them to toxins and makes them primary targets. Several exogenous compounds[21] such as $AlCl_3$. AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function.[22] Therefore, an elevation in serum concentration of ALT is an indication of liver damage.

In this study (Table 2), ALT and AST levels were significantly ($p<0.05$) increased from 11.91 ± 0.20 and 11.83 ± 0.40 to 59.23 ± 0.24 and 57.29 ± 0.50 respectively when rats were induced with aluminum chloride toxicity. When rats were treated with ethanol and aqueous extracts of *M. oleifera* at 100 mg/kg the levels of ALT dropped down to 11.62 ± 0.40 and 11.61 ± 0.17 respectively and the AST levels dropped to 11.39 ± 0.25 and 11.42 ± 0.15 respectively. A further increase in the concentrations of both extracts to 400 mg/kg resulted to a significant ($P<0.05$) elevation of ALT 11.93 ± 0.17 (ethanol) and 11.94 ± 0.07 (aqueous) and AST 11.77 ± 0.25 (ethanol) and 11.78 ± 0.15 (aqueous).

Alkaline phosphatase, ALP, is a plasma and endoplasmic reticulum membrane-bound enzyme. Transient increase of this enzyme may be noticeable in all types of liver problems. $AlCl_3$ initiated an increase in the level of this enzyme. But treatment with the two extracts of *M. oleidera* resulted in their decrease to 85.15 ± 4.11 (ethanol) and 84.51 ± 3.43 (aqueous) which were even lower than that of the control group (88.63 ± 2.4) but increasing their concentrations to 400 mg/kg lead to elevation to 88.63 ± 1.37 (ethanol) and 88.75 ± 1.37 (aqueous).

Table 1: Effects of *M. oleifera* ethanol and aqueous seed extract on the electrolytes of $AlCl_3$ treated rats

	K^+ (mEq/L)	Na^+ (mmol/L)	Cl^-(mmol/L)	Ca^+ (mg/dL)	Mg^+ (mg/dL)	Bi-C (mmol/L)
Group 1	4.37 ± 0.33^b	145.4 ± 8^c	105.00 ± 8^b	9.81 ± 0.50^c	2.19 ± 0.35^b	28.80 ± 3^b
Group 2	3.36 ± 0.13^a	109.5 ± 20^a	119.75 ± 8^c	7.58 ± 1.83^a	1.67 ± 0.16^a	31.00 ± 2^c
Group 3	4.37 ± 0.56^b	141.4 ± 9^c	101.60 ± 5^b	9.53 ± 1.68^c	2.16 ± 0.35^b	27.80 ± 4^b
Group 4	4.09 ± 0.80^a	135.0 ± 2^b	98.25 ± 5^a	9.13 ± 0.74^c	2.09 ± 0.31^b	25.00 ± 5^a
Group 5	4.02 ± 0.43^a	138.2 ± 2^b	98.80 ± 5^a	8.95 ± 0.80^b	1.96 ± 0.20^a	25.00 ± 6^a
Group 6	4.30 ± 0.17^a	145.0 ± 7^c	100.60 ± 6^b	9.70 ± 0.89^c	2.27 ± 0.26^b	28.20 ± 4^b
Group 7	4.30 ± 0.36^a	144.0 ± 7^c	100.00 ± 6^b	9.47 ± 1.19^c	2.25 ± 0.15^b	28.75 ± 2^b

Each value represents the mean of 5 rats ± SD.

K^+ = Potassium ion, Na^+ = Sodium ion, Cl^- = Chloride ion, Ca^+ = Calcium ion, Mg^+ = Magnesium ion, Bi-C = Bi-carbonate

Group 1: Control, Group 2: 100 mg/kg bw $AlCl_3$, Group 3: 100 mg/kg bw $AlCl_3$ + Standard Drug, Group 4: 100 mg/kg bw $AlCl_3$ + 100 mg/kg bw eth., Group 5: 100 mg/kg bw $AlCl_3$ + 100 mg/kg bw aq., Group 6: 100 mg/kg bw $AlCl_3$ + 400 mg/kg bw eth., Group 7: 100 mg/kg bw $AlCl_3$ + 400 mg/kg bw aq.

Table 2: Effects of *M. oleifera* ethanolic and aqueous seed extract on the liver function of $AlCl_3$ treated rats

	AST (IU/L)	**ALT** (IU/L)	**ALP** (IU/L)
Control	11.83 ± 0.40^b	11.91 ± 0.20^a	88.63 ± 2.4^b
100 mg/kg bw $AlCl_3$	57.29 ± 0.50^c	59.23 ± 0.24^b	100.84 ± 12.34^c
100mg/kg bw $AlCl_3$ + Standard Drug	11.70 ± 0.45^b	11.84 ± 0.27^a	88.63 ± 2.05^b
100mg/kg bw $AlCl_3$ + 100mg/kg bw eth.	11.39 ± 0.25^a	11.62 ± 0.40^a	85.15 ± 4.11^a
100mg/kg bw $AlCl_3$ + 100mg/kg bw aq.	11.42 ± 0.15^a	11.61 ± 0.17^a	84.51 ± 3.43^a
100mg/kg bw $AlCl_3$ + 400mg/kg bw eth.	11.77 ± 0.25^b	11.93 ± 0.17^a	88.63 ± 1.37^b
100mg/kg bw $AlCl_3$ + 400mg/kg bw aq.	11.78 ± 0.15^b	11.94 ± 0.07^a	88.75 ± 1.37^b

Each value represents the mean of 5 rats ± SD.
AST = Aspartaate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline Phosphatase

Conclusion

This study demonstrates the effectiveness of *Moringa oleifera* seeds against the alterations and imbalance caused by $AlCl_3$ intoxicated wistar rats. The low mortality rate observed during the experiment suggest the safety and efficacy of this plant extract in the system.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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