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Status of Some Biochemical Markers in *Sprague dawley* Rats Following Sub-Chronic Consumption of Alum- Treated Water

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Abstract
Alum is a double salt of aluminum and potassium sulfates. The aim of this study was to investigate the status of some biochemical markers in *Sprague-dawley* rats following sub-chronic consumption of alum- treated water. It consisted of 21 rats of 3–4 months old weighing roughly 122 ± 0.5 g. They were divided into three groups at random with 7 rats / group. The control group received 15 ml of distilled water daily for 30 days, while experimental groups one and two received 15ml of 0.04% concentration of alum-treated water for 15 days daily and 30 days respectively. Following this, 5 ml of blood specimen was withdrawn via cardiac puncture and introduced into corresponding lithium heparin anti-coagulated bottles. These specimens were spun at 1,500 revolutions per minute for 10 minutes using an 800 D macro centrifuge to extract the plasma that were utilized for the measurement of alanine aminotransferase, aspartate aminotransferase (colorimetric method), urea (urease Berthelot method), creatinine (Jaffe reaction method) and C-reactive protein (Latex turbidimetry method). Using SPSS 23.0 to perform statistical analysis on the study's findings, it was discovered that there were no significant differences between the experimental group one and the control group for all the measured biochemical parameters ($p>0.05$). However, in comparison to the control group, the results of the experimental group two revealed significant elevations ($p<0.05$) in plasma levels of alanine aminotransferase, aspartate aminotransferase and C-reactive protein. It is concluded that the plasma concentrations of alanine aminotransferase, aspartate aminotransferase and C-reactive protein may be altered in *Sprague-dawley* rats following consumption of 15 ml of 0.04% concentration of alum- treated water for 30 days. Thus, it is recommended that this study in a well guided manner be carried out in humans to ascertain if the findings will be consistent.

Keywords: *Status, Biochemical markers, Sprague dawley rats, Sub-chronic consumption of alum- treated water*

Introduction

The chemical compound known as potassium aluminum sulphate, which is a double salt of the sulphates of aluminium and potassium, is known commercially as "alum." It has a sweet flavour and is accessible as a white powder or octahedral crystals. It is transparent and colourless (Bottomly and Bottomly, 2010). It has aluminium in it, which is neurotoxic. Because these metal ions are frequently utilized as reactants for coagulation, the water treatment process can be mostly blamed for the presence of aluminium in drinking water.

The likelihood of residual coagulants in treated water rises when the ideal physico-chemical conditions for treating raw water are not sufficiently established, putting consumers in danger (Bottomly and Bottomly, 2010). Creating drinking water is a complex process and serious issue for emerging nations, especially those in Africa's tropical regions. In places with dense populations, there are significant issues with water supply since the amount and quality of groundwater are insufficient to meet all needs for

drinkable water. Therefore, surface waters must be used to create drinkable water. Turbidity and organic debris from drinking water are frequently removed using a physico-chemical process in water treatment facilities (Edzwald and Tobiason, 1999). Clarification, filtration, and refinement are the three processes in the traditional treatment of drinking water (Guilleret *et al.,* 1990).

To clear water of undesired colour and turbidity, alum is used as a flocculant. It has been utilized for this since antiquity. Its usage, together with filtration, is customary in traditional water treatment procedures throughout the world (Malik, 2018). Aluminium ions hydrolysis quickly thus forming a range of metal hydrolysis species upon addition to water. Due to its indisputable impacts on human health, the content of the metal in water treatment facilities should be kept under control (Malik, 2018).

People have long criticized using aluminium salt as a flocculant to clean drinking water (Chao *et al.,* 2019). In 2017, the Environmental Protection Agency (EPA) proposed that the secondary maximum aluminium contamination level for potable water be between 0.05 and 0.2 mg/dm^3 (Oram, 2017). The regulation by World Health Organization (WHO) states that drinking water residual aluminium should be less than 0.2 mg/dm^3 (WHO, 2017), this regulation is even tougher in some Countries. According to research by Bachir *et al.* (2016), the permitted concentration of aluminium in water for human consumption in Poland is 0.2 mg/dm^3, while 0.1 mg/dm^3 in France, Canada, Japan, and Sweden, and 0.05 mg/dm^3 in the United States (Ruyuan *et al.*, 2015). Numerous studies support limiting the acceptable threshold in drinking water to 0.1 mg/dm^3 (Yue *et al.,* 2016). Most villagers in this part of the country continue to drink alum-treated water indiscriminately, disregarding the amount of alum present in the water, despite established findings regarding the negative effects of doing so. Therefore, it is crucial to carry out this study to examines the status of a few biochemical markers in *Sprague-dawley* rats after sub-chronic consumption of alum-treated water in order to apply the results to human.

Materials and Methods

Study area

This work was done in the Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Ethical approval

Approval was obtained from the University ethical committee and the work was done according to the National Guidelines for Animal usage in research.

Experimental design

Inclusion and exclusion criteria

The *Sprague-dawley* rats used for this study were between 3-4 months old. These rats were all males and have weight ranging from 122 ± 0.5 g. Besides, they were all apparently healthy. Those that had ill health were excluded from this study.

Animal study

This study consisted of 21 male *Sprague-dawley* rats of 3-4 months old and approximately weighing 122 ± 0.5 g. Before the commencement of the study the rats were acclimatized for two weeks in the animal house of the Department of Medical Laboratory Science of the University where they were fed with pre-mix feed and water *ad-libitum.*

Pilot study

This was done to confirm the minimum concentration of alum- treated water that can cause fifty percent death (LC_{50}) in the experimental male *Sprague-dawley* rats. Sixteen male *Sprague-dawley* rats of 3-4 months old weighing 122 ± 0.5 g randomly grouped into four, with four rats per group designated as A, B, C and D were used. Each rat was given 15 ml of 0.02 %, 0.04 %, 0.06 % and 0.08 % concentration of alum- treated water for one day respectively. After this treatment they were monitored for 24 hours for signs, symptoms and death that may result from the toxic effect of the water. The LC_{50} was obtained in group C and calculated with reference to the arithmetic method of Karber as modified by Nwachukwu *et al.* (2011).

Sub-chronic toxicity study

Twenty-one *Sprague-dawley* rats aged 3-4 months, weighing 122 ± 0.5 g were grouped into 3 as follows:

Experimental group one
This group consisted of 7 male *Sprague-dawley* rats, of 3-4 months old, weighing 122 ± 0.5g. Each of these rats was given 15 ml of 0.04 % concentration of alum treated water daily for a period of 15 days.

Experimental group two
Seven male *Sprague-dawley* rats of 3-4 months old, weighing 122 ± 0.5 g made up this group. Each of these rats was given 15 ml of 0.04 % concentration of alum treated water daily which lasted for 30 days.

Control group
The rats in this group were not given any concentration of alum- treated water to consume. These rats were given 15 ml of distilled water daily for 30 days.

Upon completion of this experiment, chloroform technique was applied in the anaesthetization of the rats. The five milliliters blood specimen withdrawn from each rat was via cardiac puncture which was used for the biochemical investigations.

Sample size determination
The resource equation method as modified by Wan and Wan (2017) was adopted.

Biochemical tests
All the reagents used for this research were commercially purchased products of Randox Laboratories Limited, United Kingdom.

Measurement of alanine aminotransferase
The colorimetric method of Randox Laboratories Limited (UK) modified by Emmanuel *et al.* (2020) was used.

Measurement of aspartate aminotransferase
The colorimetric method of Randox Laboratories Limited modified by Emmanuel *et al.* (2020) was used.

Measurement of urea
Urease Berthelot method of Randox Laboratories Limited modified by Egoro *et al.* (2022) was adopted.

Measurement of creatinine
The Jaffe reaction method of Randox Laboratories Limited modified by Egoro *et al.* (2022) was adopted.

Measurement of C-reactive protein
The latex turbidimetry method of Randox Laboratories Limited modified by Emmanuel *et al.* (2021) was utilized.

Statistical analysis
The data got were illustrated as mean and standard deviation using SPSS version 23.0 for analysis. Student's "t" test was used to express the differences between the groups with a p-value of $p < 0.05$ considered statistically significant.

Results and Discussion
In this study, the male *Sprague-dawley* rats consumed 15 ml of 0.04 % concentration of alum-treated water daily for 15 days (experimental group one), and another group of male *Sprague-dawley* rats consumed 15 ml of 0.04 % concentration of alum-treated water daily for 30 days (experimental group two).

The findings from the male *Sprague-dawley* rats that drank 15 ml of alum-treated water on a daily basis for 15 days (experimental group one) as displayed in Table 1 and indicated that there was no statistically significant differences ($p>0.05$) between the mean value / standard deviation of alanine aminotransferase (8.25 ± 0.34) U/L, when compared with the control group (8.20 ± 0.31) U/L.

The results of the male *Sprague-dawley* rats in experimental group one as displayed in Table 1 also showed no significant variations ($p>0.05$) in the mean value / standard deviation of aspartate aminotransferase (7.84 ± 0.30) U/L as compared to the control group (7.80 ± 0.28) U/L after ingesting 15 ml of alum-treated water on a daily basis for 15 days.

These biochemical results for the liver enzyme biomarkers alanine aminotransferase and aspartate aminotransferase are suggestive of normal liver status, meaning that the rats' consumption of 15 ml of 0.04 % concentrated alum-treated water for 15 days had no adverse

effects on their livers. The results of this investigation are in contradiction with the earlier research by Ighodaro *et al. (*2012) who reported significant elevations of these biochemical biomarkers.

Results for the male *Sprague-dawley* rats in experimental group one as displayed in Table 1 that drank 15 ml of 0.04 % concentrated alum-treated water daily for 15 days showed no significant differences ($p>0.05$) in the mean value / standard deviation of urea (1.89 ± 0.27) mmol/L as compared to that of the control group (1.85 ± 0.22) mmol/L.

Results for the male *Sprague-dawley* rats in experimental group one as displayed in Table 1 that drank 15 ml of 0.04 % concentrated alum-treated water every day for 15 days revealed no significant differences ($p>0.05$) in the mean value / standard deviation comparison of creatinine value (53.29 ± 1.13) µmol/L to the control group (53.25 ± 1.10) µmol/L.

These biochemical results for the renal biomarkers urea and creatinine which may be a pointer to normal kidney function, meaning that the rats' consumption of 15 ml (0.04 %) of alum-treated water for 15 days had no negative effects on their kidneys is contradictory to the earlier research by Michael and John (1989) who reported significant elevations of these biochemical markers.

The results of the male *Sprague-dawley* rats (experimental group one) that drank 15 ml of 0.04 % concentrated alum-treated water daily for 15 days revealed no statistically significant differences ($p>0.05$) in the mean value / standard deviation of C-reactive protein (4.33 ± 0.17) mg/L as shown in Table 1 compared to the control group's (4.30 ± 0.14) mg/L.

This biochemical data for the inflammatory biomarker C-reactive protein suggests that the 15 ml of 0.04 % concentrated alum-treated water that these rats drank for 15 days may not have caused an inflammatory condition. .

However, the results in the male *Sprague-dawley* rats (experimental group two) compared with that of the control group as shown in Table 2 revealed significant elevations ($p<0.05$) in the mean values / standard deviation of alanine aminotransferase (17.21 ± 0.96) U/L, aspartate aminotransferase (15.10 ± 0.86) U/L, and C-reactive protein (9.22 ± 0.75) mg/L as compared with the control group alanine aminotransferase (8.20 ± 0.31) U/L, aspartate aminotransferase (7.80 ± 0.28) U/L and C-reactive protein (4.30 ± 0.14) mg/L.

These elevated findings of alanine aminotransferase and aspartate aminotransferase which are liver enzyme biomarkers as established in this study and in agreement with the previous study of Bai *et al. (*2012) may be suggestive of injury imposed on the liver because of the bioaccumulation of aluminium in the liver due to the prolonged consumption of 15 ml of 0.04 % concentration of alum treated water for 30 days. The liver's attempt to breakdown the stored aluminium may have resulted in its oxidative injury and the release of these enzymes into the plasma while the elevated mean value of C-reactive protein results may be indicative of an inflammatory disease as revealed in this study.

There was no statistically significant difference ($p>0.05$) between the mean values / standard deviation of urea (1.90 ± 0.37) mmol/L in the experimental group two rats when compared to that of the control group (1.85 ± 0.22) mmol/L as shown further in Table 2. This finding is as established in this study. There was also no statistically significant difference ($p>0.05$) between the mean values / standard deviation of creatinine (53.30 ± 1.15) µmol/L in the experimental group two rats when compared to the control group (53.25 ± 1.10) µmol/L as expressed in Table 2 which is as established in this study as well.

The overall findings of the mean values / standard deviations of urea and creatinine biochemical markers in these experimental group two *Sprague dawley* rats may be suggestive of normal kidneys status.

Conclusion

The conclusion drawn from this study showed that consumption of 15 ml, 0.04 % concentration of alum-treated water daily for 30 days could cause *Sprague dawley* rats to develop hepato-inflammatory disorder while renal status remained unaffected. However, the *Sprague dawley* rats' hepato-renal and inflammatory

biomarkers did not respond negatively to daily consumption of 15 ml of alum-treated water with a 0.04 % concentration for 15 days.

Recommendations

(i) It is not recommended to consume alum-treated water for an extended period of time at concentrations equal to or higher than 0.04%.

(ii) Before consuming any alum treated water, the amount of alum in the water must be properly considered.

(iii) People who drink water treated with alum at a concentration equal to or higher than 0.04% should periodically visit a registered and authorized medical laboratory institution for liver panel / inflammatory condition tests.

Table 1: Results Showing the mean ± SD of Biochemical Markers in Control and Experimental Group one Sprague dawley Rats

Parameters	Control group (n= 7)	Experimental group (n= 7)	p-value	Remarks
ALT (U/I)	8.20 ± 0.31	8.25 ± 0.34	0.84	NS
AST (U/I)	7.80 ± 0.28	7.84 ± 0.30	0.81	NS
CRP (mg/L)	4.30 ± 0.14	4.33 ± 0.17	0.61	NS
Urea (mmol/L)	1.85 ± 0.22	1.89 ± 0.27	0.72	NS
Creatinine	53.25 ± 1.10	53.29 ± 1.13	0.76	NS

Values are expressed as mean ± S.D.

Keys

ALT = alanine aminotransferase
AST = aspartate aminotransferase
CRP = C-reactive protein
NS = not statistically significant
n = number of rats

Table 2: Results Showing the mean ± SD of Biochemical Markers in Control and Experimental Group two Sprague dawley Rats

Parameters	Control group (n= 7)	Experimental group (n= 7)	p-value	Remarks
ALT (U/I)	8.20 ± 0.31	17.21 ± 0.96	0.02	S
AST (U/I)	7.80 ± 0.28	15.10 ± 0.86	0.03	S
CRP (mg/L)	4.30 ± 0.14	9.22 ± 0.75	0.02	S
Urea (mmol/L)	1.85 ± 0.22	1.90 ± 0.37	0.91	NS
Creatinine	53.25 ± 1.10	53.30 ± 1.15	0.97	NS

Values are in mean ± S.D.

Keys

ALT = alanine aminotransferase
AST = aspartate aminotransferase
CRP = C-reactive protein
S = statistically significant
NS = not statistically significant
n = number of rats

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