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# Biochemistry and Histopathology Effects of Wistar Rats Fed with Water treated with alum-*Musa paradisiaca* Hybrid

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## Abstract
Water scarcity, exacerbated by factors such as overpopulation, industrialization, deforestation, agricultural practices, and climate change, poses a significant challenge in developing countries, leading to deteriorated water quality and limited access to potable water. To address this pressing issue, a potable water treatment plant was designed and fabricated while alum-*Musa Paradisiaca* hybrid was used as coagulant for the treatment of water from the Ezu River, the effects of the *alum-Musa paradisiaca* was monitored using Wistar rats. There was significant reduction ($p<0.05$) of alanine transaminase (ALT), aspartate aminotransferase (AST) and Urea values obtained from Wistar rat fed with raw Ezu water (Group A), Wistar rat fed with tap water (Group B) and Wistar rat fed with treated water while results indicates that the value of alkaline phosphatase (ALP), creatinine, total bilirubin and direct bilirubin in Wistar rat has insignificant difference ($p>0.05$) between the Ezu water (Group A), Wistar rat fed with tap water (Group B) and Wistar rat fed with treated water.

*Keywords*: **Water Treatment, Alum-*Musa paradisiaca*, Wistar rat, Kidney, Liver, Heart.**

## I. INTRODUCTION
Water is a natural resource that is important for humans and other creatures to survive on the earth (Hussein, 2016; Khezri, Mansoorian, Atabi, Moghaddam, Khanjani, and Rashtch, 2017; Singh and Saxena, 2020; Sirirerkratana, Kemacheevakul, Chuangchote, 2019; Ugwu, Umuokoro, Echiegu, Ugwuishiwu and Enweremadu, 2017). It is as such, the greatest gift of nature for the sustainability of the ecological system (Mohd-saller, Mohd-zin and Othman, 2019). Water scarcity persists in developing countries due to over population (Hakizimana, Gourich, Chafi, Stiriba, Vial, *et al.*, 2017; Khan, Khan, Ahmed, Farooqi, Dhingra, *et al.* 2019; Manilal, Harinarayanan, Nampoothiri and Soloman, 2017), urbanization (Gurung, Dahl, and Jansson, 2016), industrialization (Nair and Sreedharan 2018), deforestation, agriculture (Al-Qodah and Al-Shannag 2017) and climate change (Ebba, 2021). Presently, water quality has significantly deteriorated (Sanchez, Marin, Visscher and Rietveld, (2012) and drinking water access has become one of the major problems faced (Tukki, Barminas, Osemeahon, Onwuka and Donatus, 2016; Valverde, Paccola, Pomini, Yamaguchi and Bergamasco, 2018). The water is vulnerable to various forms of pollution generated from different sources mainly households and agriculture. Improving access to safe drinking water can result in tangible benefits to health (Kumar and Kansagara, 2014). Conventional water treatment methodologies frequently rely on synthetic chemicals, which often necessitate importation at considerable expense, thus posing a significant economic burden on developing countries (Sa'id, Mohammed, Adie and Okuofu, 2016). The utilization of these synthetic chemicals introduces potential health risks, exemplified by the correlation between aluminum-based coagulants and the onset of Alzheimer's disease (Faizuneesa, Kanniyappan and Saranya, 2020).

Furthermore, the employment of synthetic chemicals in water treatment engenders the production of substantial sludge volumes, complicating environmental disposal efforts (Ali, Tiaiaa and Nasir 2019). Consequently, the imperative to mitigate the risks inherent in synthetic chemical usage necessitates the exploration of cost-effective and sustainable alternatives for water treatment, without compromising coagulation efficacy or microbiological integrity. Natural coagulants present an environmentally conscious alternative to their chemical counterparts (Odika, Nwansiobi, Nwankwo, Ekwunife and Onuoha, 2020).

The exploration of plant-based materials, specifically plantain leaves, as coagulants for reducing solids and microorganisms in polluted water has been limited, despite their potential as a readily available waste material (Premkumar et al., 2021).

Clinical biochemistry parameters are important markers of the overall health status of animals and can be used to investigate the toxicity of chemicals and drugs (Niyomcohan, Chatgat, Chatawatee, Keereekoch, Issuriya, Jaisamut et al., 2023). Literally studies explains that the enzymatic activity of alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) are used to evaluate liver malfunctions as these enzyme's levers are usually raised in acute hepatoxicity (Awotunde, Adewoye, Dhanabel and Hawumba, 2019; Ezeigwe, Ezennaya and Nwobodo, 2023). Liver function test provides information about the state of the Wistar liver by describing its functionality, cellular integrity and link with biliary tract (Arunsi, Chinyere, Ngwogu, Ngwogu. Atasie, Oti, et al., 2020). However, the increase in alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) could be either the sign of an underlying condition affecting the liver (Adeoye, Alimba and Oyeleke, 2015; Arhoghro and Kpomah, 2022; Awotunde et al., 2019; Ezeigwe et al., 2023; Ojuederie, Ajiboye and Babalola, 2020) or could be due to increase in muscular activity of the rat due to availability of more energy and protein in the diet (Ezeigwe et al., 2023). Alanine transaminase (ALT) a cytosolic enzyme whose activities increase as a result of cellular membrane damage (Arunsi, et al., 2020).

## II. MATERIALS AND METHODS

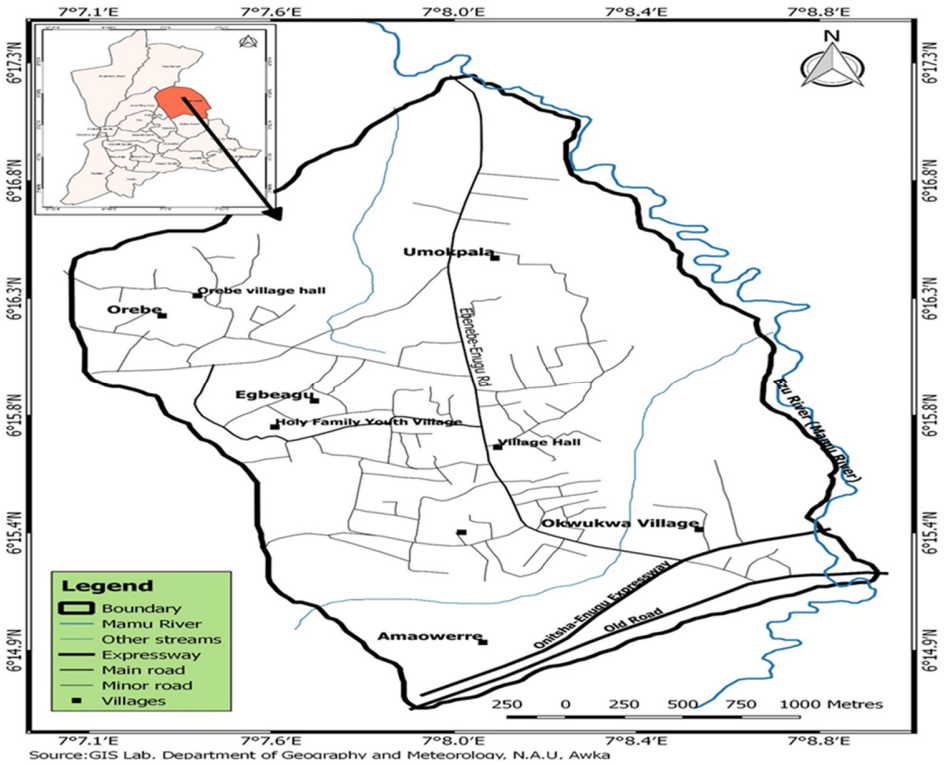
### *a) Collection of Water Sample*

The raw water sample was collected from Ezu River (Mamu River) at a location of longitude 60 15' and latitude 70 08'. Ezu river has its source at Agbogwugwu in Awgu Local Government Area of Enugu state and traverses through Amansea on Enugu-Onitsha Old Road Anambra state and continued to Ebenebe- Ugbene through Awba Offemili, all in Awka North Local Government Area, where it joins Ugwulugwu River and empties into Omambala (Anambra) River and subsequently River Niger and the Atlantic Ocean. The water sample was collected from the river by immersing a plastic container under the water (about 1 m) until it filled up and the cap inserted while the container was still underwater. The water sample was transported to the laboratory immediately in an ice pack.

### *b) Harvesting of Musa paradisiaca Leaf*

The freshly dried Musa paradisiaca leaves were harvested from a local farm at Nsukka, 9thmile corner and Emene Enugu all in Enugu State and were sent to Department of crop science, Nnamdi Azikwe University for identification. The harvested leaves were washed with distilled water to remove dirt and dried under the sun for 30days stored in a white container, before being sent to Scientific Equipment Development Institute (SEDI) Enugu for processing into ash.




*c) **Experimental animals***

For scientific research a total of 36 Wistar rats weighing between 121g-130g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State, and randomly divided into three groups of twelve rats each and used for the study. They were maintained and housed in cages under standard environmental conditions (27°C ± 3°C, 12-hour light/dark cycle) in Chris Experimental Animal Farm and Research Laboratory, Awka. The rats were weighed, marked, and put into labelled cages.

*d) **Feeding of Experimental animals***

The experimental animals were fed with fed purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State. Wistar rat were grouped into Group A, Group B and Group C, Group A were fed with raw water from Ezu river, group B were fed with tap water used as control while group C were fed with Ezu water treated with alum-*Musa paradisiaca* hybrid. The feeding was done for a period of four weeks after which the rats were fasted and anesthesized with chloroform before blood collection. Blood was collected by cardiac puncture and put in the EDTA bottles and plain bottles for hematological and biochemical analysis respectively. The carcasses were properly disposed by burying.

*e) **Liver Function Test***

Serum biochemical indices routinely estimated for liver functions were analyzed. They include Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

*f) **Kidney Function Test***

Serum biochemical indices routinely estimated for kidney functions were analyzed. They include Creatinine and urea. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

## III. Results and Discussions

### *i. Body weight of Wistar rat*

Figures 1 and Table 1 represent the growth response curve of a Wistar rat fed with different types of water over a period of 28 days,

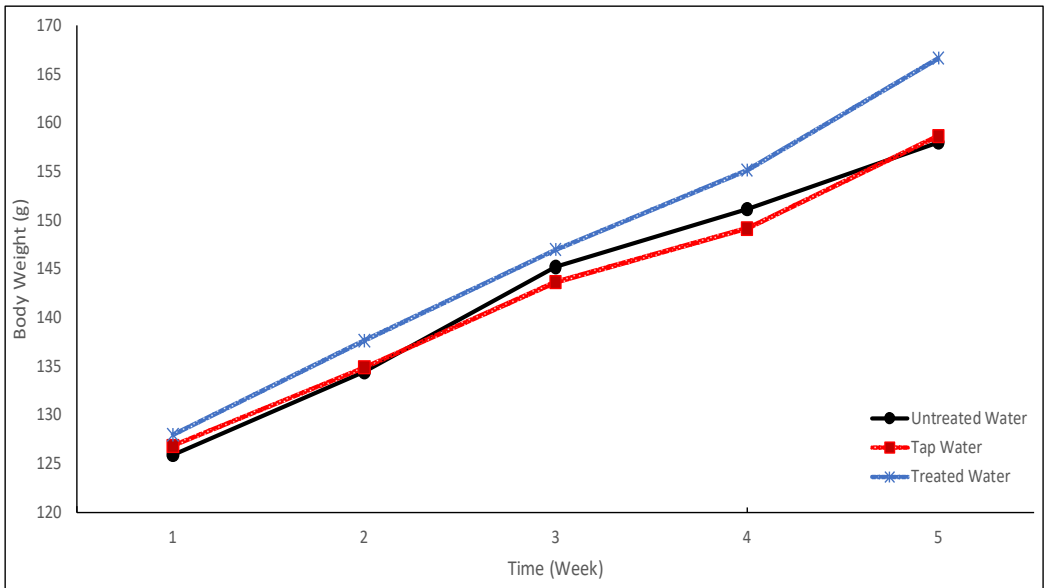



Figure 1: Body Weight of Wistar Rat Fed with Different Types of Water

The average body weight of all the Wistar rat after birth were 125.92g 126.83g and 128g for group A, group B and group C respectively. It was noted that there are statistically significant differences ($p<0.05$) between the weight of Wistar rat in the three different groups and also the weight of Wistar rat measured on a weekly basis. It was observed that Wistar rat fed with Ezu water treated with alum-*Musa paradisiaca* hybrid (Group C) has more weight than Wistar rat fed with raw water from Ezu river (Group A) and tap water (Group B).

### *ii. Clinical Biochemistry Analysis of Wistar Rat.*

Figures 2 to 8 and Tables 2 to 4, shows the weekly clinical biochemistry analysis of Wistar rat. Alanine transaminase, a crucial enzyme predominantly found in the liver, serves as a key indicator of hepatocellular integrity, and its fluctuations in serum levels can provide valuable insights into liver health

Table 1: Body weight of Wistar rats fed with different types of Water

| Duration | Group A (g) | Group B (g) | Group C (g) |
|---|---|---|---|
| Week 0 | 125.92 | 126.83 | 128.00 |
| Week 1 | 134.42 | 134.92 | 137.67 |
| Week 2 | 145.22 | 143.67 | 147.00 |
| Week 3 | 151.17 | 149.17 | 155.17 |
| Week 4 | 158.00 | 158.67 | 166.67 |

(Suciu, Abenavoli, Pellicano, Luzza, and Dumitraşcu, 2020). The observed ALT levels in Wistar rats exposed to raw Ezu river water, tap water, and Ezu water treated with an alum-*Musa paradisiaca* hybrid offer a comparative analysis of the potential hepatotoxic effects associated with these water sources (Sharma, Suri, Chandana, Singha, Sattib, *et al.*, 2016). The initial ALT

values on day 7 provide a baseline assessment of the immediate impact of the different water sources on liver function, with the rats consuming raw Ezu river water exhibiting an ALT level of 4 u/l, those consuming tap water showing 8 u/l, and those consuming treated Ezu water displaying 6.7 u/l. The subsequent measurements on days 14, 21, and 28 allow for the evaluation of the long-term effects and potential adaptive responses of the liver to the respective water treatments. The use of Wistar rats in this study is strategic due to their well-characterized physiology and sensitivity to environmental stressors, making them a suitable model for assessing the potential toxicity of water sources (Abu, Onoagbe and Ekugum 2022). The observed increase in ALT levels over time, specifically from day 14 to days 21 and 28, suggests a potential cumulative effect of the water sources on hepatic function. The consistent elevation of ALT levels in rats consuming tap water indicates a possible presence of contaminants or additives in the tap water supply that may exert a degree of hepatotoxicity (Elgaml, Khalil, Hashish and El-murr, 2015). This finding underscores the importance of regular monitoring and quality control of tap water sources to ensure their safety for consumption. The fluctuating ALT levels in rats consuming raw Ezu river water and treated Ezu water suggest a more complex interaction between the water's constituents and the liver's metabolic processes. It was observed that there is significant reduction ($p<0.05$) between alanine transaminase (ALT) values obtained from Wistar rat fed with raw Ezu water (Group A), Wistar rat fed with tap water (Group B) and Wistar rat fed with treated water. Alkaline phosphatase (ALP) is an enzyme in the cells lining of the biliary duct of the liver, osteoblasts of the bone, cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta (Aliyu and Samaila, 2016). A rise in plasma alkaline phosphatase (ALP) level is usually a characteristic finding in cholestatic liver disease

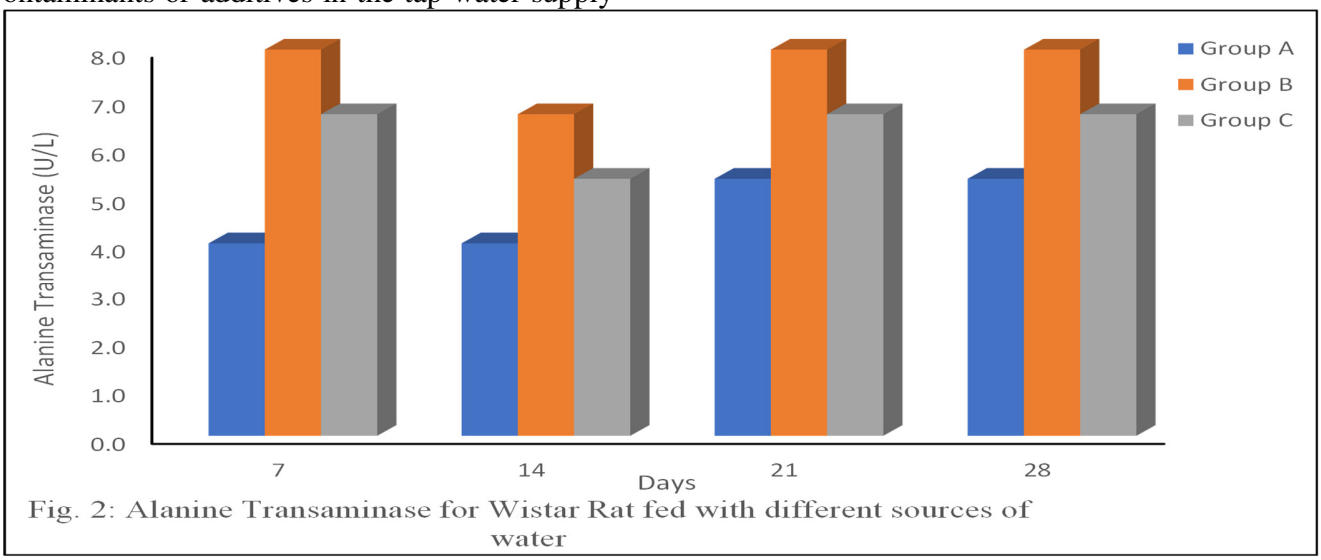



Fig. 2: Alanine Transaminase for Wistar Rat fed with different sources of water

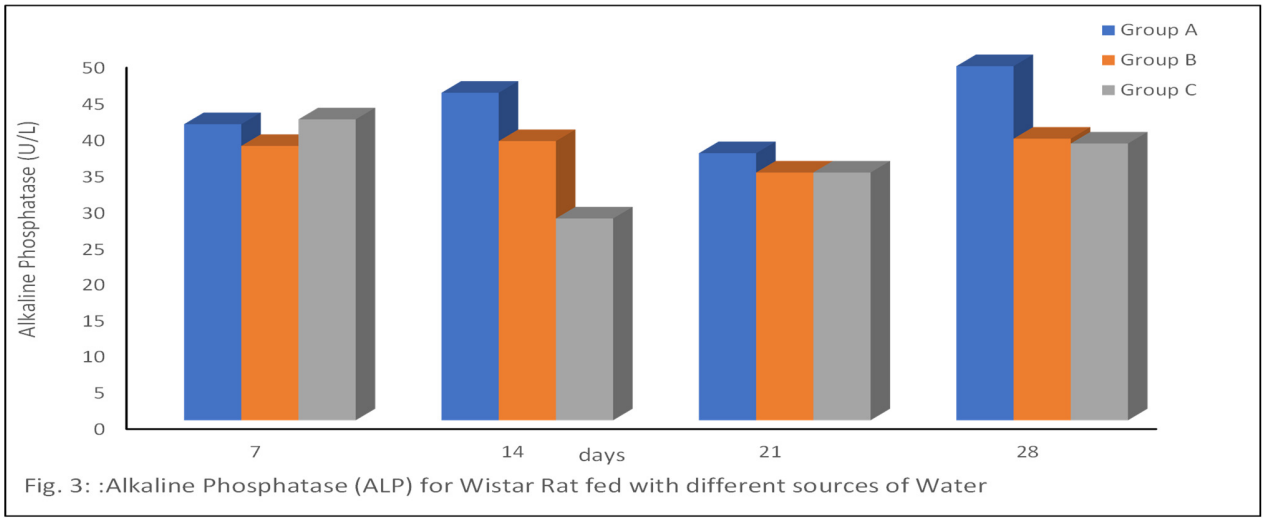


Fig. 3: :Alkaline Phosphatase (ALP) for Wistar Rat fed with different sources of Water

(Aliyu and Samaila, 2016; Ekakitie, Orororo and Okpoghono, 2022; Imafidon and Okunrobo, 2012; Wang, Dong, Zhang, Zhou, Zhou, Wee et al., 2019). On day 7, the alkaline phosphatase values were recorded as 41 u/l, 38 u/l, and 41.7 u/l for Groups A, B, and C, respectively, indicating a relatively similar baseline level of enzyme activity across all three groups at this early stage of the experiment. As the experiment progressed to day 14, the alkaline phosphatase values shifted to 45.3 u/l, 38.7 u/l, and 28 u/l for Groups A, B, and C, respectively, suggesting a possible divergence in enzyme activity due to the different water sources (Sharma et al., 2013). Notably, Group C, which received the treated water, exhibited a lower alkaline phosphatase level compared to the other two groups, potentially indicating a beneficial effect of the alum-*Musa paradisiaca* hybrid treatment in reducing factors that may elevate alkaline phosphatase activity (Cheng and Zhao, 2023). By day 21, the alkaline phosphatase values were 37 u/l, 34.3 u/l, and 34.3 u/l for Groups A, B, and C, respectively, showing a convergence in enzyme activity between Groups B and C, while Group A maintained a slightly higher level. The convergence of alkaline phosphatase levels in Groups B and C at day 21 could suggest an adaptation of the Wistar rats to the water sources or a delayed effect of the treatment method. At day 28, the alkaline phosphatase values were 49 u/l, 39 u/l, and 38.3 u/l for Groups A, B, and C, respectively, revealing a notable increase in enzyme activity in Group A, while Groups B and C maintained relatively stable levels. The observed increase in alkaline phosphatase in Group A at day 28 could be indicative of a cumulative effect of the raw Ezu River water on liver or bone health, warranting further investigation into the specific factors present in the water that may be contributing to this elevation. The results indicates that the value of alkaline phosphatase (ALP) in Wistar rat has insignificant difference ($p>0.05$) between the groups A, B and C. Aspartate Aminotransferase (AST) is an enzyme found in high levels in the liver, heart and muscles. They are good markers of damage to liver cells (Opeymi, Olubunmi, Olayumoke, Oladayo, Abiola, Bolanle, *et al.*, 2019). They are normally presents at a low level in the blood so if the liver cells are damaged, it will be expected that some of the enzymes will leak into the blood and increase the levels. Figure 4, Tables 2 shows the weekly value of Aspartate Aminotransferase (AST) in Wistar rats fed with different sources of water. The observed fluctuations in aspartate aminotransferase levels across the three groups of Wistar rats throughout the 28-day experimental period provide a compelling narrative regarding the hepatotoxic effects of raw Ezu River

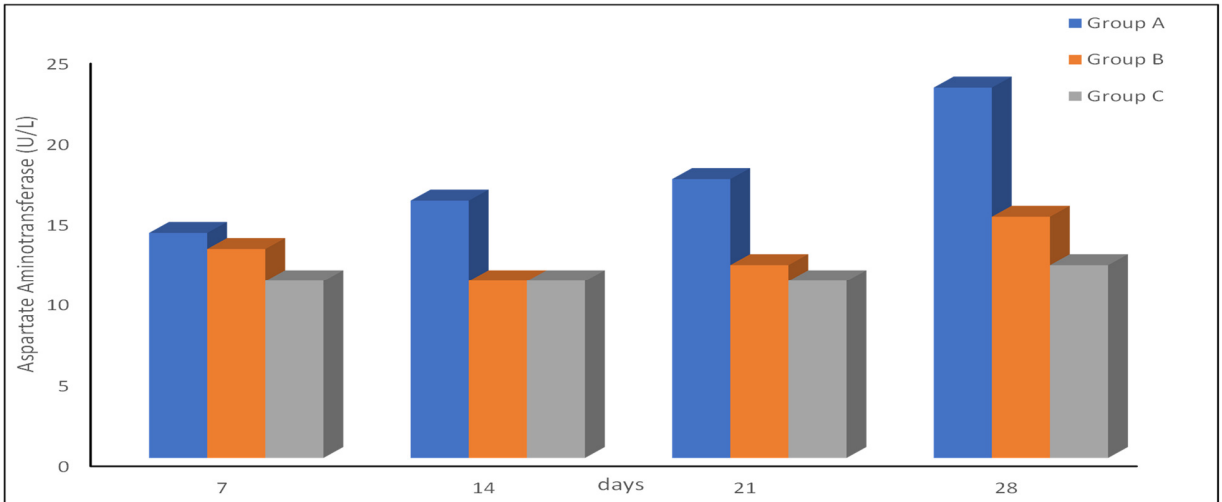


Fig. 4: Aspartate Aminotransferase (AST) for Wistar Rat fed with different sources of water

Water and the potential protective role of the alum-*Musa paradisiaca* hybrid treatment. On day 7, the AST levels in Group A were recorded at 14 u/l, suggesting an early indication of hepatic stress induced by the contaminants present in the raw water, while Group B (tap water) exhibited a slightly lower AST level of 13 u/l, indicative of normal hepatic function in the absence of significant waterborne toxins. In contrast, Group C, receiving the treated water, displayed the lowest AST level of 11 u/l, suggesting that the alum-*Musa paradisiaca* hybrid treatment might have effectively reduced the concentration of hepatotoxic substances in the water, thereby minimizing liver damage. By day 14, a discernible trend emerged, with Group A showing a further increase in AST levels to 16 u/l, reinforcing the hypothesis of ongoing hepatic injury due to continuous exposure to the contaminated water; Group B maintained a relatively stable AST level of 11 u/l, consistent with their exposure to clean tap water; interestingly, Group C sustained their low AST level of 11 u/l, further supporting the efficacy of the treatment in mitigating hepatic damage The AST levels in Group A progressively increased to 17 u/l by day 21, indicating a cumulative effect of the waterborne toxins on the liver; Group B continued to exhibit stable AST levels at 12 u/l, affirming the absence of hepatic stress; and Group C maintained the lowest AST levels at 11 u/l, corroborating the sustained protective effect of the treatment. Serum urea and creatinine concentrations are measured to assess renal function and diagnose renal diseases. Serum urea and creatinine levels would be higher than normal when the renal filtration rate is significantly decreased due to various renal disease (Arusi, *et al*., 2020; Du, Zhang, Zheng, Nie, Zhang, Feng, *et al*., 2023).

Creatinine derived from creatine is released into the plasma at a constant rate and is freely filtered by the glomerulus but not reabsorbed or metabolized by the kidney. Over time creatinine levels have been locally used to mirror the kidney function of an organism but these levels are substantially affected by the nutritional status, muscle mass, age and sex of the organism (Ezeigwe, *et al*., 2023). The value of serum urea and creatinine in Wistar rat fed with different sources of water were presented in figures 5 and figures 6. On day 7, the creatinine levels in Group A (raw Ezu river water) were recorded at 12 u/l, while Group B (tap water) showed a level of

13 u/l, and Group C exhibited a level of 14 u/l. These initial measurements provide a baseline for comparison and evaluation of changes over the subsequent weeks of the study. By day 14, the creatinine levels were 12 u/l, 10 u/l, and 14 u/l for Groups A, B, and C, respectively. The slight decrease in creatinine level observed in Group B, which received tap water, could indicate a relatively stable renal function compared to the other groups. On day 21, the creatinine levels were 12 u/l for Group A, 11 u/l for Group B, and 15 u/l for Group C. These values suggest a potential increasing trend in creatinine levels in Group C, which received Ezu water treated with alum-*Musa paradisiaca* hybrid, compared to the other groups. Finally, on day 28, the creatinine levels were 12 u/l for Group A, 12 u/l for Group B, and 16 u/l for Group C. The gradual increase in creatinine levels in Group C warrants further investigation to determine the underlying cause and assess the long-term implications of the water treatment method on renal health. The consistency of creatinine levels in Group A (raw Ezu river water) and Group B (tap water) over the 28-day period suggests that these water sources did not significantly impact renal function in the short term, though long-term effects might differ (Lou, Cheng, Liang, and Xia 2021).

Serum urea, a common biomarker, reflects the kidney's ability to filter waste products from the bloodstream, providing insights into the overall health and functional capacity of the renal system (Adebola, Oluwatoyin, Toyin and Linda, 2021). The results from Figures 6, revealed fluctuations in serum urea levels across the experimental groups and time points, indicating the dynamic nature of renal response to varying water treatments (Nayohan, Susanto, Wiryawan and Jayanegara, 2021). Specifically, on day 7, the serum urea values were recorded as 9 u/l for rats in Group A (raw Ezu river water), 10 u/l for rats in Group B (tap water), and 11 u/l for

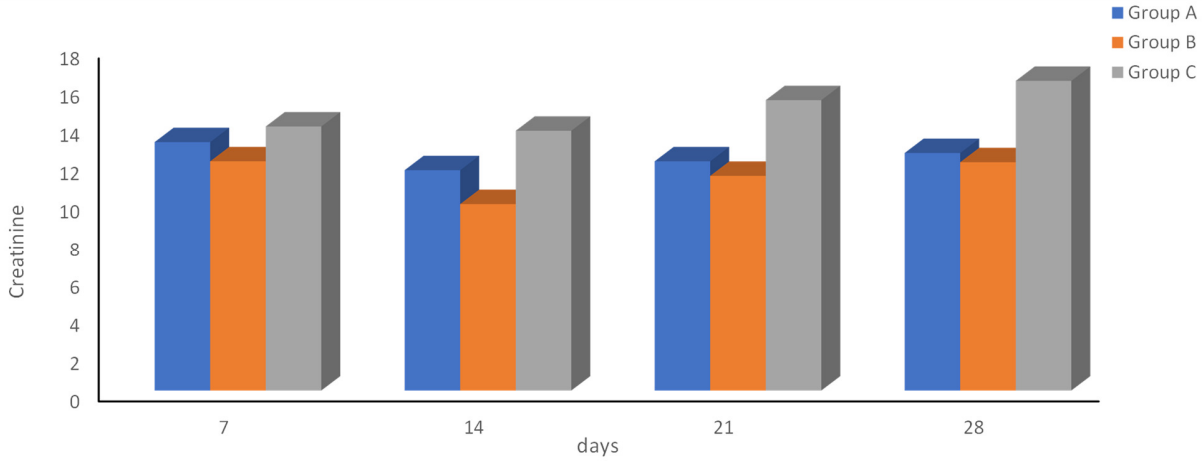


Fig. 5: Creatinine for Wistar Rat fed with different Source of Water

Table 2: Clinical Biochemistry Analysis of Wistar Rat fed with different Sources of Water

| | Alanine Transaminase (U/L) | | | Alkaline phosphatase (U/L) | | | Aspartate Aminotransferase (U/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| Days | Group A | Group B | Group C | Group A | Group B | Group C | Group A | Group B | Group C |
| 7 | 4.0 | 8.0 | 6.7 | 41.0 | 38.0 | 41.7 | 14 | 13 | 11 |
| 14 | 4.0 | 6.7 | 5.3 | 45.3 | 38.7 | 28.0 | 16 | 11 | 11 |
| 21 | 5.3 | 8.0 | 6.7 | 37.0 | 34.3 | 34.3 | 17 | 12 | 11 |
| 28 | 5.3 | 8.0 | 6.7 | 49.0 | 39.0 | 38.3 | 23 | 15 | 12 |

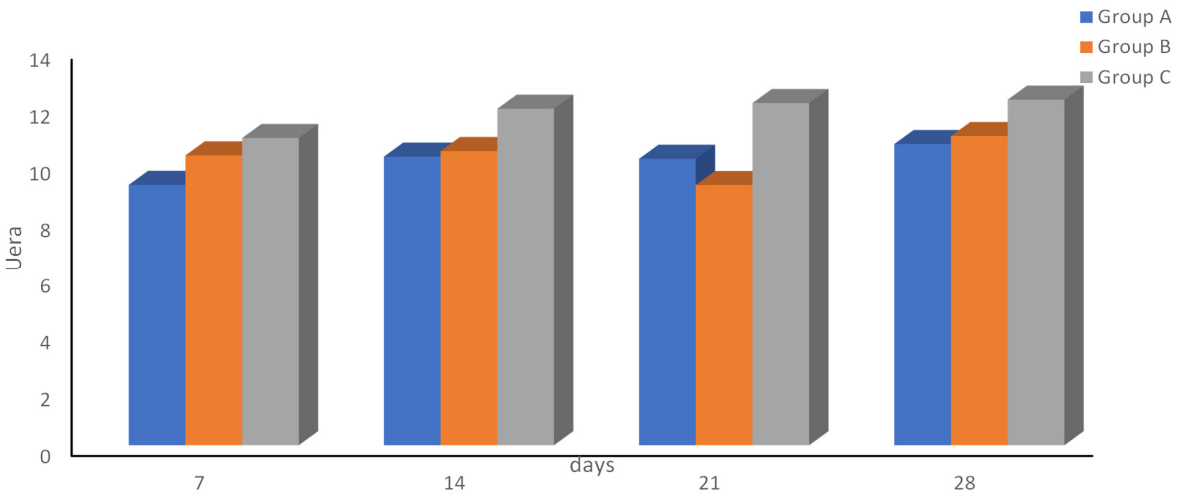


Fig. 6: Urea for Wistar rat fed with different Source of Water

rats in Group C (Ezu water treated with alum-*Musa paradisiaca* hybrid). By day 14, the serum urea levels shifted to 10 u/l, 10 u/l, and 12 u/l for Groups A, B, and C, respectively, which indicated a slight increase in Groups A and C while Group B remained constant. The values at day 21 showed a stable level in Group A at 10 u/l, a slight decrease in Group B to 9 u/l, and a consistent level in Group C at 12 u/l (Baracho, Kangussu, Prestes, Silveira, Pereira et al., 2016). Lastly, at day 28, serum urea levels were 11 u/l, 11 u/l, and 12 u/l for Groups A, B, and C, respectively, demonstrating a convergence in Groups A and B and consistent level in Group C, which suggests that the observed changes may not be drastic, it warrant careful consideration in the context of overall renal health and potential long-term effects. The observed variations in serum urea levels among the groups may be attributed to several factors, including the presence of nephrotoxic substances in the raw Ezu river water, the relative purity of tap water, and the potential impact of the alum-*Musa paradisiaca* hybrid treatment on water quality. The potential presence of pollutants or contaminants in the raw Ezu river water could induce renal stress, leading to altered urea filtration and excretion (Chinnappan, George, Krishnamurthy, Choudhary, Choudhary et al., 2019). In contrast, tap water, which is typically subjected to purification processes, may exert less stress on the kidneys, resulting in relatively stable serum urea levels (Humudat and Al-Naseri, 2020). Furthermore, the alum-Musa paradisiaca hybrid treatment, intended to improve water quality, could potentially introduce its own set of compounds that influence renal function, thereby contributing to the observed differences in serum urea concentrations (Aa et al., 2017). It was observed that there was significant difference ($p<0.05$) of Urea in group A (Wistar rat fed with raw Ezu water) group B, (Wistar rat fed with tap water) and group C, (Wistar rat fed with treated water) while there are increasingly insignificant difference ($p<0.05$) of creatinine found in group A (Wistar rat fed with raw Ezu water) group B, (Wistar rat fed with tap water) and group C, (Wistar rat fed with treated water). Bilirubin (formerly referred to as haematoidin) is the yellow breakdown product of normal haemecatabolism (Odiegwu, Chianella, Azubuike, Odiegwu and Ogbuowelu, 2021). The demonstration of the potent antioxidant activity of bilirubin, has led to the hypothesis that bilirubin's main physiologic role is a cellular antioxidant. Bilirublin is excreted in bile and urine and elevated levels may

indicate certain disease such as Jaundice, the neurotoxicity of neonatal hyper bilirubinemia etc. (Odiegwu *et al.*, 2021). Figures 7 and 8 represents the mean value of direct bilirubin and total bilirubin respectively. Direct bilirubin, a water-soluble form of bilirubin, is typically elevated in cases of liver damage or biliary obstruction, making it a valuable marker for evaluating liver function and overall health (Dongmo, Epoh, Tadjoua, Yousuf, Telefo *et al.*, 2019). On day 7, the direct bilirubin levels were relatively similar across all three groups, with values of0.7 u/l, 0.7 u/l, and 0.8 u/l for Groups A, B, and C, respectively. This initial similarity suggests that any potential hepatotoxic effects of the raw Ezu River water had not yet manifested or were within the normal physiological range for these animals. However, as the experiment progressed, notable differences emerged in direct bilirubin levels, particularly in Group A, which consumed raw Ezu River water. By day 14, the direct bilirubin level in Group A decreased slightly to 0.5 u/l, while Group B exhibited a level of 0.9 u/l, and Group C, receiving treated water, showed a level of 0.5 u/l. This decrease in Group A at day 14 requires careful consideration, as it could indicate an adaptation of the rats to the contaminants in the Ezu River water or a temporary fluctuation in bilirubin metabolism. The divergence in direct bilirubin levels became more pronounced by day 21, with Group A exhibiting a level of 1.1 u/l, Group B at 0.8 u/l, and Group C at 0.9 u/l. This increase in Group A suggests a cumulative effect of the raw water on liver function, potentially indicating the onset of hepatotoxicity. The most significant differences were observed on day 28, where Group A demonstrated a direct bilirubin level of 1.4 u/l, while Group B had a level of 1.1 u/l, and Group C had a level of 1.0 u/l.

Table 3: Clinical Biochemistry Analysis of Wistar Rat fed with different Sources of Water

| Days | Creatinine (U/L) | | | Serum Urea (U/L) | | |
|---|---|---|---|---|---|---|
| | Group A | Group B | Group C | Group A | Group B | Group C |
| 7 | 13 | 12 | 14 | 13 | 12 | 14 |
| 14 | 12 | 10 | 14 | 12 | 10 | 14 |
| 21 | 12 | 11 | 15 | 12 | 11 | 15 |
| 28 | 12 | 12 | 16 | 12 | 12 | 16 |

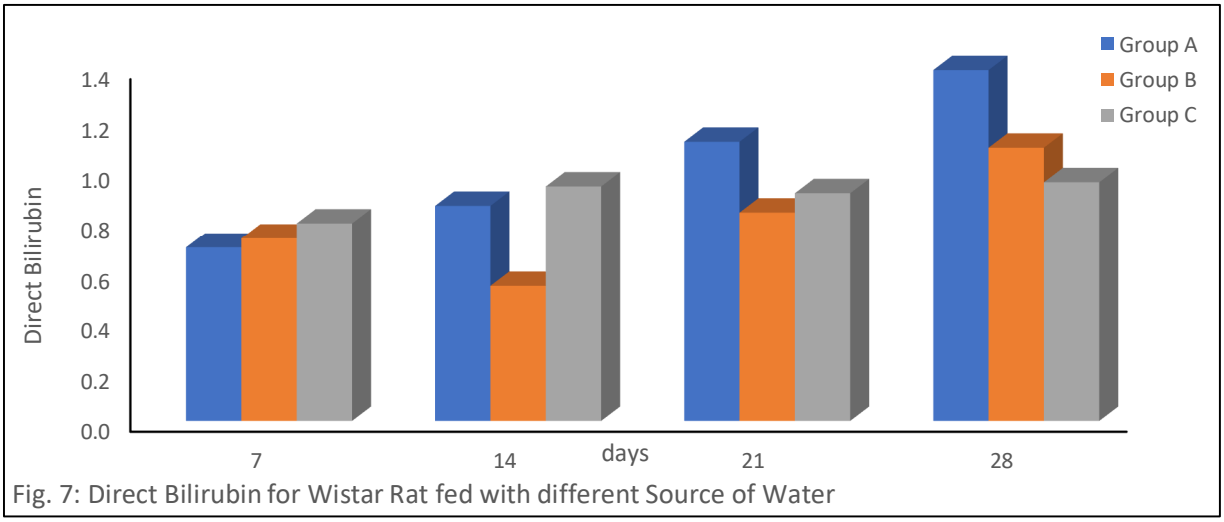


Fig. 7: Direct Bilirubin for Wistar Rat fed with different Source of Water

The elevated direct bilirubin level in Group A on day 28 strongly suggests that prolonged exposure to raw Ezu River water induces hepatic damage, leading to impaired bilirubin metabolism and excretion. The fact that group C, which was administered alum-*Musa paradisiaca* hybrid

treated Ezu water, showed reduced levels of direct bilirubin suggests that this treatment approach was successful (Kim, Yang, Lee, Park, Hong *et al.*, 2014). The consistently lower direct bilirubin levels in Group C compared to Group A indicate that the alum-*Musa paradisiaca* hybrid treatment is effective in mitigating the hepatotoxic effects of Ezu River water. The investigation into total bilirubin levels in Wistar rats subjected to varying water sources reveals a complex interplay of environmental factors and physiological responses (Abeni, Petrera, Pra, Rapetti, Crovetto et al., 2018). On day 7, rats in Group A, consuming raw Ezu river water, exhibited a total bilirubin level of 2.68 u/l, while Group B, receiving tap water, showed a level of 2.05 u/l, and Group C, provided with Ezu water treated with an alum-*Musa paradisiaca* hybrid, registered 1.79 u/l. These initial values suggest a potential impact of water source on bilirubin metabolism, with raw river water possibly imposing a greater metabolic burden on the rats (Baracho *et al.*, 2016). By day 14, the total bilirubin levels shifted, with Group A at 2.91 u/l, Group B at 1.74 u/l, and Group C at 2.05 u/l, indicating a dynamic adaptation to the respective water sources over time. The fluctuations observed between day 7 and day 14 underscore the importance of longitudinal monitoring in toxicological studies, as single time-point measurements may not fully capture the adaptive responses of the organism (Zucker, Horn, and Sherman., 2004). At day 21, the values converged, with Group A at 2.34 u/l, Group B at 2.54 u/l, and Group C at 2.57 u/l, suggesting a possible homeostatic regulation of bilirubin levels despite the continued exposure to different water sources. However, by day 28, the total bilirubin levels diverged again, with Group A exhibiting 3.17 u/l, Group B at 2.32 u/l, and Group C at 2.45 u/l, potentially indicating a cumulative effect of the raw river water on bilirubin metabolism (Ruiz, Crespo, Martínez, Iruzubieta, Casals *et al.*, 2021). The persistent elevation in Group A's bilirubin levels at day 28 could signify sub-acute toxicity (Dongmo *et al.*, 2019).

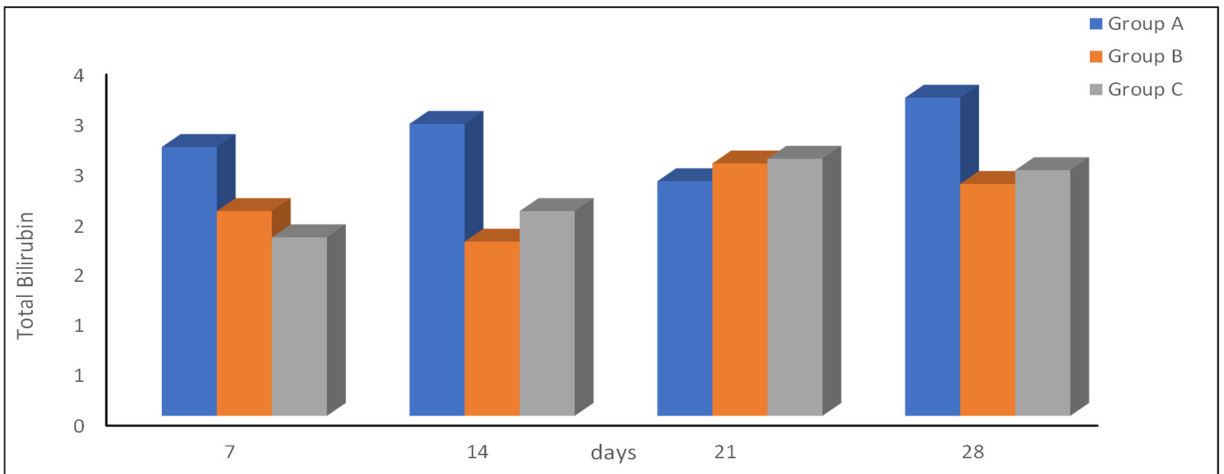


Fig. 8: Total Bilirubin for Wistar Rat fed with different Source of Water

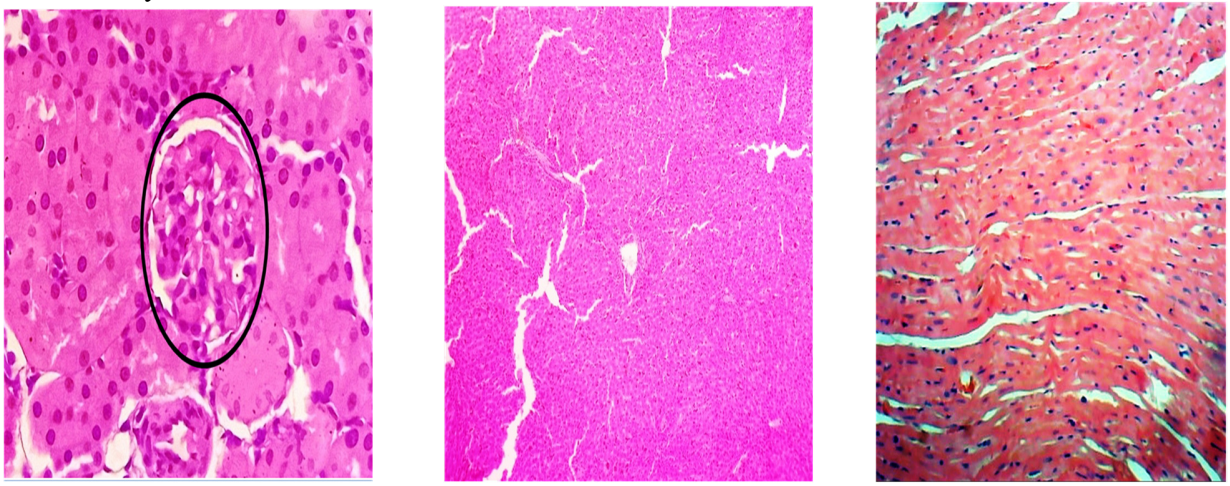
  

Table 4: Clinical Biochemistry Analysis of Wistar Rat fed with different Sources of Water

| Days | Direct Bilirubin (U/L) | | | Total Bilirubin (U/L) | | |
|---|---|---|---|---|---|---|
| | Group A | Group B | Group C | Group A | Group B | Group C |
| 7 | 0.7 | 0.7 | 0.8 | 2.68 | 2.05 | 1.79 |
| 14 | 0.9 | 0.5 | 0.9 | 2.91 | 1.74 | 2.05 |
| 21 | 1.1 | 0.8 | 0.9 | 2.34 | 2.52 | 2.57 |
| 28 | 1.4 | 1.1 | 1.0 | 3.17 | 2.32 | 2.45 |

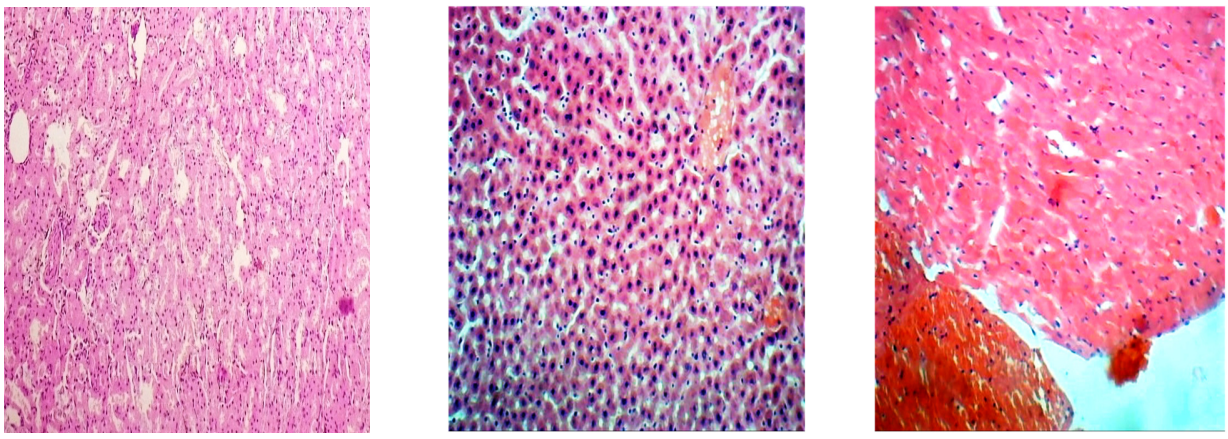
iii. Histopathological analysis of the Liver, Kidney and Heart.

A histopathology evaluation was carried out to ascertain the toxicity of the water treated with Alum-*Musa paradisiaca* hybrid and also to identify the structural changes. Plates 1 to 3 shows the light micrographs of the liver, kidney and the heart.



**(a)** **(b)** **(c)**

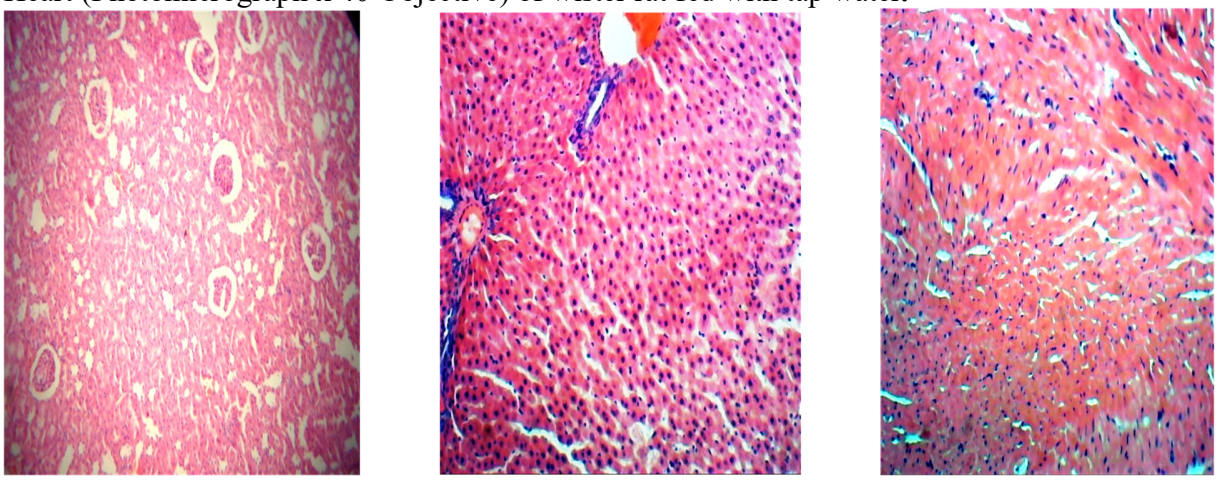
Plates 1: Histological sections of kidney, liver and heart **(a)** Kidney (Photomicrograph x 40 Objective) of wister rat fed with raw water from Ezu river. **(b)** Liver (Photomicrograph x 10 Objective) of wister rat fed raw water from Ezu river. **(c)** Heart (Photomicrograph x 40 Objective) of wister rat fed raw water from Ezu river.



**(a)** **(b)** **(c)**

Plates 2: Histological sections of kidney, liver and heart **(a)** Kidney (Photomicrograph x 10 Objective) of wister rat fed with tap water. **(b)** Liver (Photomicrograph x 40 Objective) of wister rat fed with tap water. **(c)** Heart (Photomicrograph x 40 Objective) of wister rat fed with tap water**.**



**(a)** **(b)** **(c)**

Plates 3: Histological sections of kidney, liver and heart **(a)** Kidney (Photomicrograph x 20 Objective) of wister rat fed with water treated Alum-*Musa paradisiaca* hybrid. **(b)** Liver (Photomicrograph x 40 Objective) of wister rat fed with water treated Alum-*Musa paradisiaca* hybrid. **(c)** Heart (Photomicrograph x 40 Objective) of wister rat fed with water treated Alum-*Musa paradisiaca* hybrid**.**

Light microscopic examination of the vital organs including the liver, kidney and heart of the selected Wistar rat did not reveal any gross pathological lesions. The photomicrographs of the kidney, liver and heart of the Wistar rat fed with both, raw water from Ezu river (Group A), tap water which was used as control (Group B) **a**nd treated water with Alum-*Musa paradisiaca* hybrid (Group C), showed normal morphological architecture. Under microscopic examination, the kidney, liver and heart of the selected group of Wistar rats fed with water treated with Alum-*Musa paradisiaca* hybrid (Group C) showed normal architecture and binucleation and was without any distortions similarly with group of Wistar rat (Group B) fed with the tap water which was used as control.

Furthermore, sign of injury, necrosis, congestion, fatty acid accumulation or hemorrhagic regions around the central vein or sinusoids of the liver were not observed. The hepatocytes arranged in cords were clearly visible. The cross-section of the river (Group A), tap water (Group B) and Ezu water treated Alum-*Musa paradisiaca* hybrid (Group C) were essentially normal. There were also no lesions (pathological changes) in the tissue of the animals. liver showed no lyses in the blood cells or infiltration of neutrophil, lymphocyte or macrophage in the subacute oral toxicity.

As for liver the kidneys histologically, there was no morphological change for the Wistar rats fed with water treated with Alum-*Musa paradisiaca* hybrid (Group C). The appearance of the glomerular architecture was normal similar to group of Wistar rat (Group B) fed with the tap water which was used as control. The glomeruli, distal and proximal tubules in the kidney appeared normal in all the groups. In addition, there was no interstitial and intraglomerular congestion or tubular atrophies. All the nephron cells were normal and showed clearly visible nucleoli with no degeneration bleeding, necrosis or infiltration with lymphocytes. The hearts showed normal cardiac muscle fibers for all the groups.

From the present study, it was shown that the tissue section of kidney, liver and heart of a Wistar rat fed with raw water from Ezu

Thus, the histopathological evaluation of the selected organs did not reveal any morphological abnormalities that could be attributed to the feeding

of the Wistar rat with Ezu water treated with Alum-*Musa paradisiaca* hybrid.

## IV. Conclusion.

From the present study, it can be concluded that the liver, kidney and heart have no sign of injury, necrosis, congestion, fatty acid accumulation and as such no morphological changes or abnormalities could be attributed to the feeding of Wistar rat with Ezu water treated with Alum-*Musa paradisiaca* hybrid.

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