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Original Research Article

Ameliorative effects and histological assessment of active fractions of ethanolic fruits extract of *Raphia hookeri* on $AlCl_3$ induced toxicity in rats

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ABSTRACT

Background: Aluminium chloride is a widely distributed element with a well-established toxicity. The study aimed at evaluating ameliorative effects of active fractions and ethanolic fruits extracts of *Raphia hookeri* on $AlCl_3$-induced toxicity in male rats. The objectives included; determination of liver and kidney function biomarkers, lipid profile, histopathological assessment of the organs.
Methods: A total of 110 healthy male rats weighing 180-200 g were grouped into 11 groups of 10 rats each. Group 1: Normal feed and water (normal control). Group 2: $AlCl_3$ only. Group 3: 200 mg/kg b. w of vitamin C. Group 4 and 5: N-hexane fraction at 10 and 20 mg/kg b. w. Group 6 and 7: ethyl acetate fraction at 10 and 20 mg/kg b. w. Group 8 and 9: Aqueous fractions at 10 and 20 mg/kg b. w. Group10 and 11: Ethanol extract at doses of 200 mg/kg b. w and 400 mg/kg b. w. The treatment lasted for 21 days.
Results: Results revealed a significant ($p<0.05$) decreased in the activities of ALT, AST, ALP, TB, DB and TP. It further revealed a significant ($p<0.05$) decrease in urea, creatinine, sodium, potassium and chloride. Also, a significant ($p<0.05$) decrease in CHOL, TG, HDL-C, LDL-C was observed. Histopathological assessment of the liver and kidney tissues corroborated the observed changes in enzymes activities.
Conclusions: The findings demonstrated ameliorative potentials of active fractions and ethanolic fruit extract of *Raphia hookeri* against hepatic and renal damage induced by $AlCl_3$ in a dose and time dependent manner.

Keywords: *Raphia hookeri*, Liver function, Kidney function, Lipid profile, Histopathological assessment

INTRODUCTION

Aluminum (Al) is one of the most abundant metallic elements on the planet. The availability of Al has recently drawn more attention to its biotoxicity.[1,2] Al is used as a food additive, in cooking pots with roughly 20% Aluminum content, in drinking water with a 0.2-mg/l concentration, as a water-purifying agent, in can bottles, in aluminum foil paper, and in antiperspirant cosmetic products. Although having a low gastrointestinal absorption capacity (less than 1%), it may accumulate over time in vital organs like the kidney, liver, and brain, where it may cause apparent neurotoxicity and cytotoxicity.[3]

Additionally, Al may stimulate the pro-oxidant features of iron and copper, which results in mitochondrial dysfunction, the oxidative degradation of macromolecules, and the release of cytochrome C from the mitochondria.[4] Therefore, eliminating Al toxicity through neutralization and scavenging of free radicals may be a viable option. *Raphia hookeri* (Rh) commonly known as Raphia palm belongs to the Family: Palmaceae is a monocotyledon plant, commonly found in West Africa and very abundant in lowlands and swampy areas in north-central part of Nigeria where, it grows in water to about 1 m deep.[5,6] Raffia palm roots have a variety of medicinal uses. In traditional medicine, the root extract is used to prepare a laxative and to alleviate stomach pain in infants.[7] Various experimental models have investigated the effects of Raffia palm (*Raphia hookeri*) leaf extract on enzymes linked to type-2 diabetes mellitus (T2DM) and pro-oxidant induced oxidative stress in rat pancreas. Not much is known about the fruit (mesocarp) though; it is used as a forest food. In view of this and in the context of continuous search for safe treatment of oxidative stress diseases affecting the vital organs such as the liver and the kidney this study therefore, sought to investigate the ameliorative effects and histological assessment of active fractions and ethanolic fruits extract of *Raphia hookeri* in $AlCl_3$-induced toxicity in rats.

METHODS

The research was conducted from November 2022 to October 2023. All the samples were analysed at Federal Medical Centre, Makurdi, Benue State, Nigeria.

Experimental animals

Total of 110 Wistar rats were kept in cages under standard laboratory conditions (25 ^{0}C), 12-h light/12-h dark cycle and had free access to grower mash, Vital Feeds Company Nigeria and clean tap water *ad-libitum* for two (2) weeks prior to the commencement of the experiment according to the guidelines of the organisation for economic cooperation and development.[8] The experimental animals were randomized into eleven (11) groups of ten (10) rats in each group.

Drugs and chemicals

Aluminum Chloride ($AlCl_3$) was obtained from Sigma-Aldrich Co. (USA). All other chemicals and kits were of highest analytical grade.

Experimental design

Total of 110 healthy male Wistar rats weighing 180-200 g were grouped into 11 groups of 10 rats each. Aluminium chloride 4.2 mg/kg b. w was administered intraperitoneally once to all the experimental animals except group 1. Group 1 normal control rats received normal feed and water. Group 2 $AlCl_3$ control received $AlCl_3$ 4.2 mg/kg b. w intraperitoneally once without treatment. Group 3 received $AlCl_3$ 4.2 + 200 mg/kg b. w of vitamin C. Group 4 and 5 received $AlCl_3$ 4.2 + n-hexane fraction at 10 and 20 mg/kg b. w. Group 6 and 7 received $AlCl_3$ 4.2 + ethyl acetate fraction at 10 and 20 mg/kg b. w. Group 8 and 9 received $AlCl_3$ 4.2 + aqueous fractions at 10 and 20 mg/kg b. w. While group10 and 11 received $AlCl_3$ 4.2 + ethanolic extract at doses of 200 mg/kg b. w and 400 mg/kg b. w.

Collection of serum samples

After 24 hours of last/final administration of the fractions and ethanolic fruit extracts of *R. hookeri*, the experimental animals were starved overnight, anaesthetized with chloroform and sacrificed, about 2 mL of blood sample was collected through cardiac puncture, and was separated into EDTA for plasma and plain bottles for serum respectively. The serum was obtained by centrifuging the samples at 3000 rpm for 5 minutes using bench top centrifuge (MSE minor, England). The serum samples were frozen in a freezer until required.

Evaluation of serum biochemical indices

Liver function parameters: Serum assay of Liver function biomarkers such as alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, and total protein levels were determined as described by Chriatjanti et al.[9] Lipid profile: Total cholesterol (CHOL), triglycerides (TG), high density lipoprotein (HDL-CHOL), and low density lipoprotein (LDL-CHOL) concentrations were determined according the method adopted by Arowora et al and Uhegbu et al.[10,11] Kidney function parameters: Serum kidney function biomarker such as urea, creatinine, sodium, potassium and chloride were determined as described by Ge et al Imo et al.[12,13]

Histopathological examination of hepatic and renal tissues

The histological examination hepatic and renal tissue was accomplished using the modified methods adopted by Titford et al, Mohammed et al.[14,15] The animals were anaesthetized in chloroform using a desiccator. The rats were dissected, and the liver and kidneys harvested and observed for evidence of gross pathology. The organs were quickly separated and fixed in 10% buffered formalin. The tissues were sectioned at a thickness of 5 µm using an automated tissue processor (LeicaModelRM2125) bathed in paraffin, and stained with hematoxylin and eosin (H&E). The microscopic architecture of experimental rats on the H&E stained slides was histologically examined where photomicrographs were taken using a Motic 9.0 Megapixels Microscope Camera at x100 and x400 magnifications respectively.

Statistical analysis

Statistical analysis was carried out with One way Analysis of Variance (ANOVA) and further with Duncan's multiple

comparison using Statistical Package for the Social Sciences (SPSS), version 26.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean±SEM (n=5), and difference between groups considered significant at $p<0.05$.

RESULTS

Effect of active fractions and ethanolic fruit extract of R. hookeri on liver function biomarkers of $AlCl_3$ induced toxic rats

ALT revealed a significant ($p\leq0.05$) increase in the negative control **(**104.80±2.58) while revealing significant ($p\leq0.05$) decrease in all the treatment groups with $AlCl_3$ + Eth 10 mg (16.09±1.88) having the highest decrease. There was however, non-significant ($p\leq0.05$) difference when the following treatment groups; $AlCl_3$ + Vit. C 200 mg (21.83±0.86) $AlCl_3$ + 20 mg aq (24.05±2.06) when compared with the normal control group (23.74±1.68). AST revealed significant ($p\leq0.05$) decrease in the treated groups when compared with the negative control (133.53±2.80), treatment group $AlCl_3$ + 20 mg aq (23.01±4.51) revealed highest level of decrease. ALP revealed a significant ($p\leq0.05$) decrease in all treatment groups when compared with the negative control group (188.96±3.61) with treatment group $AlCl_3$+ Crude 200 mg (44.78±4.82) revealing the highest level of decrease. TB revealed a significant ($p\leq0.05$) decrease in all the treated groups when compared with the negative control group (1.68±0.19).

DB also revealed a significant ($p\leq0.05$) decrease in all the treatment groups when compared with the negative control group (0.74±0.17) the highest decrease was observed in group; AlCl3 + n-h 10 mg (01±0.00), AlCl3 + Eth 10 mg (0.02±0.00), AlCl3+Eth 20 mg (0.04±0.01), AlCl3+10 mg aq (0.02±0.00), AlCl3 + Crude 200 mg (0.05±0.01), AlCl3 + Crude 400 mg (0.04±0.01) when compared with the normal control group (0.33±0.10). TP significantly ($p\leq0.05$) increased in AlCl3 + n-h 20 mg (42.24±0.59) when compared with the negative control (5.90±1.28). GLU significantly ($p \leq0.05$) increased in AlCl3 + Crude 400 mg (0.47±0.02), AlCl3 + 10 mg aq (0.40±0.00), AlCl3 + Eth 20 mg (0.39±0.03), and AlCl3 + Crude 400 mg (0.5±0.06) when compared with the negative control (0.32±0.00). CHOL revealed a significant ($p\leq0.05$) decrease in all the treatment groups when compared with the negative control group (580.90±8.23). TGL revealed a significant ($p\leq0.05$) decrease in all the treated groups when compared to the negative control group (2.65±0.25). However, there was non- significant ($p\leq0.05$) difference in all the treatment groups AlCl3 + n-h 10 mg (0.71±0.01), AlCl3 + n-h 20 mg (0.69±0.03), AlCl3 + Eth 10 mg (0.70±0.03), AlCl3 + Eth 20 mg (0.66±0.01), AlCl3 + 10 mg aq (0.82±0.05), AlCl3 + 20 mg aq (0.77±0.06) and AlCl3 + Crude 400 mg (0.72±0.01) except AlCl3+ Crude 200 mg (0.09±0.02) when compared to the normal control group (0.84±0.02). HDL-CHOL also revealed a significant ($p\leq0.05$) decrease in all the treatment groups when compared to the negative control group (4.41±0.34). Non-significant ($p\leq0.05$) difference was observed in all the treated groups; AlCl3 + n-h 10 mg (3.26±0.11), AlCl3 +n-h 20 mg (3.30±0.06), AlCl3 + Eth 10 mg (3.19±0.18), AlCl3 + 10 mg aq (3.23±0.07), AlCl3 + 20 mg aq (3.44±0.03), AlCl3+ Crude 200 mg (3.35±0.00), AlCl3 + Crude 400 mg (3.33±0.00) except in AlCl3+Eth 20 mg (2.42±0.53) when compared to the normal control group (3.70±0.04). LDL-CHOL revealed a significance ($p\leq0.05$) decrease in all treatment groups when compared to the negative control (1.99±0.21). However, there was a non-significance ($p\leq0.05$) difference in all treatment groups when compared to the normal control (0.12±0.0).

Effect of active fractions and ethanolic fruit extract of R. hookeri on kidney function biomarkers of $AlCl_3$-induced toxic rats

The Urea levels revealed a significant ($p\leq0.05$) decrease in all treatment groups when compared with the negative control group (1.50±0.18). Cr also revealed the same significant ($p\leq0.05$) decrease when all the treatment groups were compared with the negative control (0.19±0.00). Na+ revealed a significant ($p\leq0.05$) decrease in AlCl3 + Eth 20 mg (3.28±0.04), AlCl3 + Eth 10 mg (3.23±0.00), AlCl3 + 10 mg aq (3.22±0.02), AlCl3 + n-h 10 mg (3.15±0.06), AlCl3 + 20 mg aq (3.01±0.03), AlCl3 + Crude 200 mg (2.88±0.00) when compared to the negative control group (5.50±0.33).

K^+ revealed a significant ($p\leq0.05$) difference when all treatment groups were compared with the negative control (2.67±0.24). CL revealed a significant ($p\leq0.05$) decrease in $AlCl_3$+10 mg aq (1.86±0.02), $AlCl_3$+ Crude 200 mg (1.85±0.02), $AlCl_3$+Eth 20 mg (1.80±0.22), $AlCl_3$ + Eth 10 mg (1.75±0.02), $AlCl_3$ + 20 mg aq (1.65±0.17), $AlCl_3$ + Crude 400 mg (1.61±0.06), $AlCl_3$ + n-h 20 mg (1.53±0.09), $AlCl_3$ + n-h 10 mg (1.28±0.04), when compared to the negative control group (5.02±0.38).

Histological effect of active fractions and ethanolic fruit extract of R. hookeri on the kidney of $AlCl_3$-induced toxic rats

Kidney tissue of group1 (normal control) shows intact glomerulus with podocytes, convoluted tubules and cyto-architectural lining in place. Kidney tissue of 2 (negative control) rats show multifocal glomerular nephritis noticed in mild congestion of the glomerulus. Kidney tissue of group 3 shows multifocal glomerular nephritis as well as interstitial nephritis and community of cell as circled among others. Kidney tissue of group 4 shows glomerular nephritis as shown in the label (GM) below, otherwise intact cell architecture. Kidney tissue of group 5 shows glomerular nephritis, other cell structures remain intact. Kidney tissue of group 6 shows multifocal glomerular nephritis (GM) and interstitial nephritis (arrows) and community of cells (triangle) noticed. Kidney tissue of group 7 shows mild multifocal nephritis as shown by a shift in the glomerulus, cell architectures remain intact.

Table 1: Effect of active fractions and ethanolic fruit extract of *R. hookeri* on liver function biomarkers of $AlCl_3$-induced toxic rats.

Groups	ALT (U/I)	AST (U/I)	ALP (U/I)	TB (mg/dl)	DB (mg/dl)	TP (gm/dl)
Normal control	23.74±1.68	32.77±0.97	60.36±1.79	0.65±0.02	0.33±0.10	31.47±3.87
Negative control ($AlCl_3$)	104.80±2.58	133.53±2.80	188.96±3.61	1.68±0.19	0.74±0.17	5.90±1.28
$AlCl_3$ + Vit. C (200 mg)	21.83±0.86	26.61±3.55	58.71±2.52	0.20±0.06	0.32±0.13	34.94±2.41
$AlCl_3$ + n-h 10 mg	25.93±1.86	34.86±4.80	50.28±2.15	0.42±0.11	0.01±0.00	34.72±1.52
$AlCl_3$ + n-h 20 mg	24.81±1.15	28.52±3.52	57.02±3.45	0.25±0.01	0.25±0.06	42.24±0.59
$AlCl_3$ + Eth 10 mg	16.09±1.88	39.49±2.83	48.44±2.92	0.46±0.09	0.02±0.00	32.79±1.45
$AlCl_3$ + Eth 20 mg	25.42±1.38	27.57±2.49	49.67±2.30	0.57±0.09	0.04±0.01	32.86±0.93
$AlCl_3$ + 10 mg aq	30.52±1.95	24.34±1.63	52.41±3.62	0.56±0.14	0.02±0.00	25.71±0.73
$AlCl_3$ + 20 mg aq	24.05±2.06	23.01±4.51	47.08±2.91	0.87±0.04	0.13±0.06	30.72±1.21
$AlCl_3$ + Crude 200 mg	24.51±2.83	29.65±4.17	44.78±4.82	0.72±0.01	0.05±0.01	36.00±2.21
$AlCl_3$ + Crude 400 mg	24.70±2.17	22.42±4.70	43.40±6.05	0.46±0.11	0.04±0.01	32.39±0.84

Values are expressed as mean±SEM; N = 5. Values with different superscript down the column are considered statistically significant ($p \leq 0.05$) $AlCl_3$ only = Aluminium chloride without treatment. $AlCl_3$ + Vit. C 200 mg = Aluminium chloride and Vitamin C. $AlCl_3$ + n-h 10 mg = Aluminium chloride and n-hexane 10 mg. $AlCl_3$ + n-h 20 mg = Aluminium chloride and n-hexane 20 mg. $AlCl_3$ + Eth 10 mg = Aluminium chloride and ethyl acetate 10 mg. $AlCl_3$ + Eth 20 mg = Aluminium chloride and ethyl acetate 20 mg. $AlCl_3$ + 10 mg aq = Aluminium chloride and 10 mg aqueous. $AlCl_3$ + 20 mg aq = Aluminium chloride and 20 mg aqueous. $AlCl_3$ + crude 200 mg = Aluminium chloride and 200 mg crude extract. $AlCl_3$ + crude 400 mg = Aluminium chloride and 400 mg crude extract.

Table 2: Effect of active fractions and ethanolic fruit extract of *R. hookeri* on lipid profile of $AlCl_3$-induced toxic rats.

Groups	CHOL (mmol/l)	TG (mmol/l)	HDL-CHOL (mmol/l)	LDL-CHOL (mmol/l)
Normal control	249.86±1.84	0.84±0.02	3.70±0.04	0.12±0.00
Negative control ($AlCl_3$ only)	580.90±8.23	2.65±0.25	4.41±0.34	1.99±0.21
$AlCl_3$ + Vit. C (200 mg)	164.51±4.26	0.71±0.03	3.33±0.01	0.12±0.01
$AlCl_3$ + n-h 10 mg	214.67±2.80	0.71±0.01	3.26±0.11	0.15±0.03
$AlCl_3$ + n-h 20 mg	223.66±0.67	0.69±0.03	3.30±0.06	0.11±0.02
$AlCl_3$ + Eth 10 mg	201.01±5.18	0.70±0.03	3.19±0.18	0.10±0.00
$AlCl_3$ + Eth 20 mg	202.96±2.61	0.66±0.01	2.42±0.53	0.07±0.01
$AlCl_3$ + 10 mg aq	176.10±1.22	0.82±0.05	3.23±0.07	0.10±0.01
$AlCl_3$ + 20 mg aq	189.92±8.37	0.77±0.06	3.44±0.03	0.12±0.01
$AlCl_3$ + Crude 200 mg	162.86±0.04	0.09±0.02	3.35±0.00	0.23±0.10
$AlCl_3$ + Crude 400 mg	170.09±0.08	0.72±0.01	3.33±0.00	0.18±0.04

Values are expressed as mean±SEM; N = 5. Values with different superscript down the column are considered statistically significant ($p \leq 0.05$) $AlCl_3$ only = Aluminium chloride without treatment. $AlCl_3$ + Vit. C 200 mg = Aluminium chloride and Vitamin C. $AlCl_3$ + n-h 10 mg = Aluminium chloride and n-hexane 10 mg. $AlCl_3$ + n-h 20 mg = Aluminium chloride and n-hexane 20 mg. $AlCl_3$ + Eth 10 mg = Aluminium chloride and ethyl acetate 10 mg. $AlCl_3$ + Eth 20 mg = Aluminium chloride and ethyl acetate 20 mg. $AlCl_3$ + 10 mg aq = Aluminium chloride and 10 mg aqueous. $AlCl_3$ + 20 mg aq = Aluminium chloride and 20 mg aqueous. $AlCl_3$ + crude 200 mg = Aluminium chloride and 200 mg crude extract. $AlCl_3$ + crude 400 mg = Aluminium chloride and 400 mg crude extract.

Kidney tissue of group 8 shows mild interstitial spaces and community of cells as circled with intact glomerulus and cells architecture. Kidney tissue of group 9 with mild nephritis and inflammation noticed in the glomerulus and as circled with intact cell lining. Kidney tissue of group 10 shows multifocal glomerular nephritis and interstitial nephritis as shown in arrows. Kidney tissue of group 11 shows multifocal glomerular nephritis and community of cells as shown in circles A & B.

Table 3: Effect of active fractions and ethanolic fruit extract of *R. hookeri* on kidney function biomarkers of $AlCl_3$-induced toxic rats.

Groups	Urea (mmol/l)	Creatinine (mg/dl)	Na+ (mmol/l)	K+ (mmol/l)	Chloride (mmol/l)
Normal control	0.04±0.00	0.20±0.00	3.60±0.15	1.29±0.02	1.38±0.02
Negative control($AlCl_3$ only)	1.50±0.18	1.58±0.23	5.50±0.33	2.67±0.24	5.02±0.38
$AlCl_3$ + Vit. C (200 mg)	0.02±0.01	0.19±0.00	3.38±0.02	1.22±0.05	1.40±0.02
$AlCl_3$ + n-h 10 mg	0.04±0.02	0.21±0.02	3.15±0.06	1.37±0.01	1.28±0.04
$AlCl_3$ + n-h 20 mg	0.04±0.01	0.20±0.01	3.32±0.02	1.24±0.04	1.53±0.09
$AlCl_3$+ Eth 10 mg	0.02±0.00	0.25±0.02	3.23±0.00	1.05±0.03	1.75±0.02
$AlCl_3$ + Eth 20 mg	0.01±0.00	0.21±0.00	3.28±0.04	1.80±0.22	1.80±0.03
$AlCl_3$ + 10 mg aq	0.01±0.00	0.18±0.01	3.22±0.02	1.01±0.03	1.86±0.02
$AlCl_3$ + 20 mg aq	0.02±0.00	0.16±0.00	3.01±0.03	1.16±0.02	1.65±0.17
$AlCl_3$ + Crude 200 mg	0.02±0.00	0.18±0.01	2.88±0.00	1.37±0.00	1.85±0.02
$AlCl_3$ + Crude 400 mg	0.03±0.00	0.18±0.01	3.12±0.01	1.52±0.16	1.61±0.06

Values are expressed as mean±SEM; N = 5. Values with different superscript down the column are considered statistically significant $p \leq 0.05$ $AlCl_3$ only = Aluminium chloride without treatment. $AlCl_3$ + Vit. C 200 mg = Aluminium chloride and Vitamin C. $AlCl_3$ + n-h 10 mg = Aluminium chloride and n-hexane 10 mg. $AlCl_3$ + n-h 20 mg = Aluminium chloride and n-hexane 20 mg. $AlCl_3$ + Eth 10 mg = Aluminium chloride and ethyl acetate 10 mg. $AlCl_3$ + Eth 20 mg = Aluminium chloride and ethyl acetate 20 mg. $AlCl_3$ + 10 mg aq = Aluminium chloride and 10 mg aqueous. $AlCl_3$ + 20 mg aq = Aluminium chloride and 20 mg aqueous. $AlCl_3$ + crude 200 mg = Aluminium chloride and 200 mg crude extract. $AlCl_3$ + crude 400 mg = Aluminium chloride and 400 mg crude extract.

Photomicrographs of liver of $AlCl_3$- induced toxic rats

Liver tissue of normal control rats (Group1) shows hepatocytes and sinusoids dilating towards a clear central portal vein with intact cell arrangements. Liver tissue of negative control rats (Group 2) shows inflammation of cells around the portal trait as circled, necrosis of cells also noticed. Liver tissue of group 3 shows necrosis of cells and dilation of hepatocytes and sinusoids. Liver tissue of group 4 shows restoration of cells with a mild inflammation around the portal trait as circled. Liver tissue of group 5 shows normal cell architecture but inflammation of the PV and inflammatory cells as shown arrows. The liver tissue of group 6 shows ballooning degeneration, other structures remain intact. Liver tissue of group 7 shows mild inflammatory cells showing in the arrows, otherwise, normal architectural lining are all directing to the CPV. Liver tissue of group 8 shows significant restoration of the disorder. e. g no inflammation of the portal as circled. Liver tissue of group 9 shows restoration of cells with mild portal congestion due to pressure, diverting the blood flow. Otherwise, all cell architecture remains intact. Liver tissue of group 10 shows normal cell architecture with mild degeneration and few inflammatory cells noticed. Liver tissue of group 11 shows restoration structures around SH & SN but with minimal necrosis.

DISCUSSION

Aluminum is commonly found all over in the crust of the earth as one of the most abundant elements; it constitutes about 8% of all the mineral elements. It can easily gain access to the body system because it is a component of cooking utensils and medications including anti-acids, deodorants, food additives, electrical equipment and fuel additives, so toxicity of aluminum is chiefly considered due to its availability).[16,17] Because of its great availability, Al can accumulate in the liver and kidney, all of which cause serious implications to human and animal health.[18,19] The study revealed the significant antioxidant role of ethanolic fruit extract of *Raphia hookeri* in ameliorating $AlCl_3$-induced hepatotoxicity and nephrotoxicity in wistar rats. The present study revealed that the administration of aluminum chloride was associated with an elevation in concentrations of some liver biomarkers (AST, ALP, and ALT). Ighodaro et al and Yakubu et al reported that aluminum exposure is associated with the elevation of liver enzymes in the serum.[20,21] AST, ALP, and ALT are very important liver enzymes that catalyse the transfer of α-amino groups from alanine and aspartate to the α-keto group of ketoglutaric acid, with the resultant generation of pyruvic and oxalacetic acids, which are important contributors to the citric acid cycle.[2,22] Administration of active fractions and crude ethanolic fruits extract of *Raphia hookeri* simultaneously with $Alcl_3$ was able to maintain steady levels in ALT, AST, and ALP activities. This may be achieved by the high total antioxidant activity, total phenolic content and total flavonoid content as reported already.[23] Flavonoids are the most represented family of phenolic compounds. They have a good antioxidant activity through several mechanisms of action, such as inhibition of peroxidation of membrane lipids and maintaining cellular membrane integrity via neutralizing free radicals thus prevented the leakage of hepatic enzymes.[3,23] This has been related to their complex structure compared to that of phenolic acids. The elevated concentrations of total cholesterol and triglycerides in $Alcl_3$-treated rats may be attributed to the disturbance in lipid metabolism due to Al exposure.[24-26] That is consistent with Al Eisa et al who attributed the increase in the serum

cholesterol concentration in rats given $AlCl_3$ to hepatic dysfunction and the elevated serum TG concentration to the hypoactivity of lipoprotein lipase which is responsible for triglycerides degradation.[1]

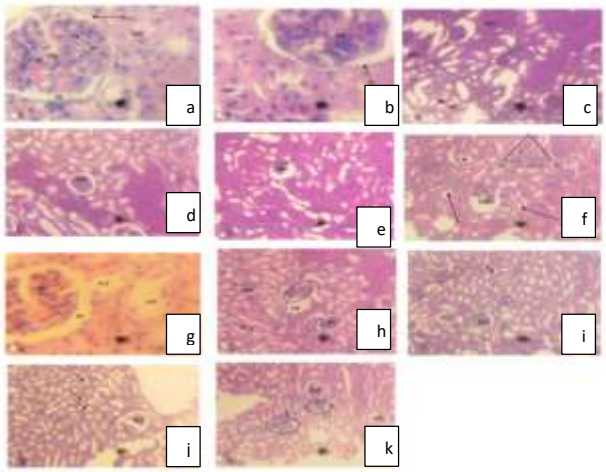

Figure 1: Photomicrographs of kidney of $AlCl_3$-induced toxic rats; a): Photomicrograph of Kidney tissue of normal control rats (Group 1) with intact glomerulus with podocytes, convoluted tubules and cyto-architectural lining in place. GM= Glomerulus PCT= Proximal convoluted tubules BS= Bowman's space BC= Bowman's corpuscle PCT= Convoluted tubules DCT= Distal Convoluted tubules (x400) b): Photomicrograph of Kidney tissue of negative control rats (Group 2) with multifocal glomerular nephritis noticed in mild congestion of the glomerulus (x400) c): Photomicrograph of kidney tissue of group 3 with multifocal glomerular nephritis as well as interstitial nephritis and community of cell as circled among others (x100) d): Photomicrograph of kidney tissue of group 4 with glomerular nephritis as shown in the label (GM) above, otherwise intact cell architecture e): Photomicrograph of kidney tissue of group 5 with glomerular nephritis, other cell structures remain intact(x100) f): Photomicrograph of kidney tissue of group 6 with multifocal glomerular nephritis (GM) and interstitial nephritis (arrows) and community of cells (triangle) noticed (x100) g): Photomicrograph of kidney tissue of group 7 with mild multifocal nephritis as shown by a shift in the glomerulus. Cell architectures remain intact (x400) h): Photomicrograph of kidney tissue of group 8 with mild interstitial spaces and community of cells as circled with intact glomerulus and cells architecture (x100) i): Photomicrograph of Kidney tissue of group 9 with mild nephritis and inflammation noticed in the glomerulus and as circled with intact cell lining (x100) j): Photomicrograph of Kidney tissue of group 10 with multifocal glomerular nephritis and interstitial nephritis as shown in arrows (x100) k): Photomicrograph of kidney tissue of group 11 with multifocal glomerular nephritis and community of cells as shown in circles A & B (x100).

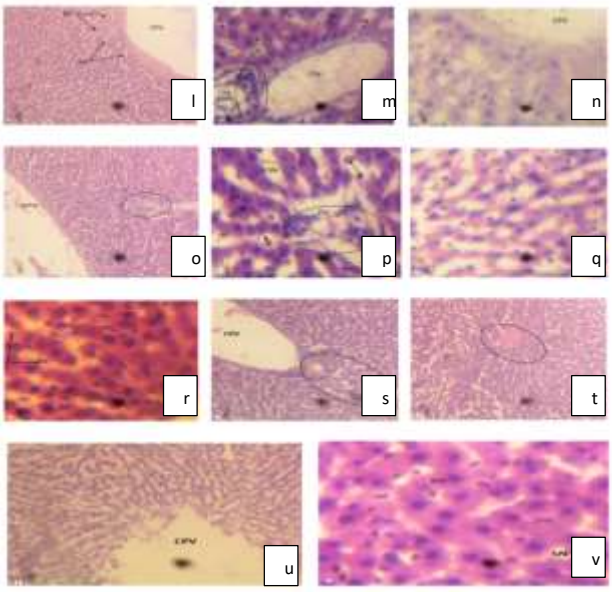

Figure 2: Photomicrographs of liver of $AlCl_3$- induced toxic rats; l): Photomicrograph of liver tissue of normal control rats (Group1) with hepatocytes and sinusoids dilating towards a clear central portal vein with intact cell arrangements. CPV= Central Portal Vein SN= Sinusoids SH= Sheets of hepatocytes PV= Portal vein PT= Portal trait (x100). m): Photomicrograph of liver tissue of negative control rats (Group 2) with inflammation of cells around the portal trait as circled, necrosis of cells also noticed. n): Photomicrograph of liver tissue of group 3 with necrosis of cells and dilation of hepatocytes and sinusoids (x400). o): Photomicrograph of liver tissue of group 4 with restoration of cells with a mild inflammation around the portal trait as circled. p): Photomicrograph of liver tissue of group 5 with normal cell architecture but inflammation of the PV and inflammatory cells as shown arrows (x400). q): Photomicrograph of liver tissue of group 6 with ballooning degeneration (x400). r): Photomicrograph of liver tissue of group 7 with mild inflammatory cells showing in the arrows, otherwise, normal architectural lining are all directing to the CPV (x400). s): Photomicrograph of liver tissue of group 8 with significant restoration of the disorder. e. g no inflammation of the portal as circled (x100). t): Photomicrograph of liver tissue of group 9 with restoration of cells with mild portal congestion due to pressure, diverting the blood flow. Otherwise, all cell architecture remains intact (x100). u): Photomicrograph of liver tissue of group 10 with normal cell architecture with mild degeneration and few inflammatory cells noticed. v): Photomicrograph of liver tissue of group 11 with restoration structures around SH & SN but with minimal necrosis (x400).

Flavonoids, polyphenols, and phenolic acids in active fractions and crude ethanolic fruits extract of *Raphia hookeri* possess antioxidant activity may be attributed to the reduction of the serum TC and TG concentrations. The

kidneys perform lots of functions in animals such as homeostasis and acid-base balance, regulation of the balance of electrolytes in the blood, removal of waste products of metabolism, secretion of some enzymes and hormones, metabolism and osmoregulation. Any alteration or abnormality associated with the kidneys could lead to non-performance or inefficiency in carrying-out these functions by the kidney. The abnormalities associated with kidney function could be ascertained by evaluating the levels of some kidney function parameters such as blood urea, creatinine, serum electrolytes and also histological examination of the organ, among others.[13] The result of this study showed that serum urea level decreased significantly in all the groups administered active fractions and crude ethanolic fruit extract of *R. hookeri* when compared with the normal control. This significant decrease is a sign of protective effect as evidenced by the antioxidant activities exhibited by the extracts administered, thereby modulating the rate at which the kidney excretes its waste products. Urea is the final degradation product of protein and amino acid metabolism. It is synthesized in the liver from ammonia produced as a result of deamination of proteins. Filtration of urea from the blood into the urine by the renal glomeruli is the major means by which excess nitrogen is eliminated from the body. Among the renal causes of increased urea levels are acute glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, and tubular necrosis. Any type of obstruction of the urinary tract is a post-renal cause for elevated BUN levels. Other possible causes of elevated urea level are cardiac decompensation, water depletion due to decreased intake and excessive loss, increased protein catabolism and high protein diet. Imo et al had reported a non- significant increase in urea level administered crude extract of *Datura metel L*.

Serum creatinine (a blood measurement) is a nitrogenous end product of muscle creatine metabolism, is a specific indicator of glomerular function because, it is an easily measured by-product of muscle metabolism that is excreted unchanged by the kidneys.[13] The significant decrease in creatinine levels in all the groups administered active and ethanolic fruit extract of *R. hookeri* is an indication that creatinine was not retained by the kidney. Nephrotoxicity is indicated by significant elevation in serum creatinine and urea levels. Impairment of the kidney is evidenced by creatinine retention. These results are in consonance with that of Saeed et al who reported blood and Bitter melon antioxidant activities, though opposing that of Imo et al.[13,25-27] Electrolytes (sodium, potassium, chloride and bicarbonate) balance in the blood is a good indicator of how well the kidneys and heart are functioning. The determination of which electrolyte is out of balance is a baseline test in treatment. Sodium is the major cation of extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. Too much sodium (hypernatremia) or too little sodium (hyponatremia) can cause cells to malfunction, and extremes in the blood sodium levels (too much or too little) can be fatal. Potassium is the principal cation of the intracellular fluid. It is also an important constituent of the extracellular fluid due to its influence on muscle activity. Elevated potassium levels (hyperkalemia) are often associated with renal failure, dehydration shock or adrenal insufficiency. Decreased potassium levels (hypokalemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex.[28] Chloride is important in the maintenance of the cation/anion balance between intra and extra-cellular fluids. This electrolyte is essential to the control of proper hydration, osmotic pressure, and acid/base equilibrium. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction. The administration of active fractions and crude ethanolic fruits extract of *Raphia hookeri* revealed significant decrease in the concentrations of urea, creatinine in all the treatment groups against the $AlCl_3$ control group. Conversely, significant increase in levels of sodium, potassium, and chloride was observed in the $AlCl_3$ control group with concomitant decrease in all the treatment groups. Imo and Uhegbu reported that significant alteration in the concentration of these body electrolytes is indicative of poor renal functions or renal impairment.[11,13] The histology of the kidney tissues has added credence to the nephrotoxic effects of aluminium chloride toxicity. Interestingly, the active fractions and ethanol fruit extract of *R. hookeri* effectively restored these histopathological alterations to normal. Histopathological changes in renal tissues induced by aluminium chloride but reversed by the administration *Vernonia amygdali*na in male Wistar rats have been documented.[29] The histological results of the liver tissues in the present study confirmed the observations from biomarkers determined. The liver of the control group revealed normal cyto-architecture of hepatocytes with normal central veins and sinusoids dilating towards a clear central portal vein with intact cell arrangements. However, degenerative changes were observed in the photomicrograph of the aluminium chloride intoxicated group with other features such as inflammation of cells around the portal trait and necrosis of cells indicating toxicity resulting from the administration of aluminium chloride. Akpanyung et al had documented similar reports on toxicity of aluminium chloride on the liver of albino rats. Normal cellular architecture of the liver was observed in the group co-administered with $AlCl_3$ and 10 mg aq fraction of *Raphia hookeri*.[30] This was evidence of ameliorative potential of *Raphia hookeri* ethanolic fruit extract against the toxic effect of $AlCl_3$. Hepatoprotective effect of aqueous extract of *Raphia hookeri* has been reported, and the present study corroborates same.[31] Mild degenerative features were still observed in some groups implying that longer period would be required for the toxic effect of $AlCl_3$ to wear off naturally.

CONCLUSION

The present study has demonstrated ameliorative effect of the active fractions and ethanolic fruit extract of *Raphia hookeri* against $AlCl_3$-induced hepatoxicity and renal impairment. These effects were in dose and time dependent manner. The results provided experimental evidence for the ethanol medicinal use of the fruits of *Raphia hookeri* in the management of oxidative stress diseases.

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