

## HEPATORENAL PROTECTIVE POTENTIAL OF RUTIN IN ALUMINIUM CHLORIDE-INDUCED OXIDATIVE STRESS IN WISTAR RAT

Olu Israel Oyewole  Ayoola Mary Bukoye 

Department of Biochemistry, Osun State University Faculty of Basic and Applied Sciences, Osogbo, Nigeria

Aluminium is a metal that disrupts both the pro-oxidant and antioxidant balance in tissues, leading to systemic biochemical dysfunction. The present study evaluated the protective effect of rutin against aluminium chloride (AlCl<sub>3</sub>)-induced hepatorenal toxicity in Wistar rats. Twenty male Wistar rats were divided into four groups: Group 1- control; Group 2 received AlCl<sub>3</sub> (100 mg/kg b.wt); Group 3 received AlCl<sub>3</sub> (100 mg/kg b.wt) plus rutin (50 mg/kg b.wt); Group 4 received rutin (50 mg/kg b.wt). Body weight changes, liver and kidney functions, antioxidant capacities, and histopathology of the liver and kidney were measured at the end of the twenty-one-day experimental period. The obtained results indicated that AlCl<sub>3</sub> caused significant weight loss in the rats. The toxicant also disrupted liver and kidney functions, characterized by significant depletion of antioxidant levels, distortion of histoarchitecture, and elevation of serum parameters. Conversely, treatment with rutin ameliorated the observed imbalances in body weight and biochemical indices and improved the histological alterations induced by AlCl<sub>3</sub> in the liver and kidneys of the rats. The study concludes that rutin exerts protective effects on the hepatorenal structure and function and may serve as a useful nutraceutical for managing liver and kidney damage in humans.

Keywords: aluminium toxicity, antioxidants, lipid peroxidation, enzyme biomarkers

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**Correspondence to:**

Olu Israel Oyewole  
Department of Biochemistry  
Osun State University  
Faculty of Basic and Applied Sciences  
Osogbo, Nigeria  
E-mail: ioluoye@yahoo.com

## INTRODUCTION

Aluminium exposure is ubiquitous among humans and animals due to its extensive use and environmental presence. It occurs naturally in the Earth's crust as insoluble aluminosilicate (1) and in various combined forms such as aluminium chloride (AlCl<sub>3</sub>). It is found in drinking water due to water treatment processes or leaching from weathered rocks and soils (2). Its metallic properties lead to its widespread use in cooking utensils and products such as deodorants, food additives, and antacids (3). This increasing exposure has significant implications for various organs, due to its diverse routes of uptake in the body, with dietary ingestion being the most prevalent.

Oxidative damage has been claimed to occur as a result of Al toxicity due to its increased free radical production (4). It also stimulates inflammatory cytokine production and induction of endoplasmic reticulum stress in the liver and kidneys (5,6). The simultaneous damage of these organs, known as hepatorenal injury, results in severe complications, including liver cirrhosis, renal failure, and eventually systemic organ dysfunction (7).

Rutin (3,3',4',5,7-pentahydroxy flavone-3-rutinoside) is a bioflavonoid antioxidant. It is naturally abundant in various plants, such as grapes, buckwheat, tea, apples, tobacco, Forsythia, fruits, vegetables, and grains (8). It possesses a strong reactive oxygen species (ROS) scavenging activity [9], activates antioxidant enzymes, and shows potential to transport electrons, thereby reducing oxidative stress [10]. It also alleviates hepatorenal injuries caused by toxic agents, including mercuric chloride and lead acetate (11-13). As shown experimentally, rutin has anti-carcinogenic, cardioprotective, antithrombotic, and neuroprotective activities, and enhances liver health and integrity (14-16). Owing to this identified characteristic, this research focuses on hepatorenal injuries caused by Al toxicity and the potential role of rutin in alleviating oxidative damage, thereby improving liver and kidney function.

## METHODS

Rutin (rutoside) was obtained from Bulk Supplements (Nevada, USA), and analytical kits from Randox Laboratories Ltd., County Antrim, BT29 4QY, United Kingdom. Aluminium chloride and all other necessary reagents of analytical grade were obtained from Sigma-Aldrich Chemicals Co., St. Louis, MO, USA.

### Experimental design and animal grouping

Twenty male Wistar rats (average weight 140 g) were obtained and housed in netted plastic cages at the Central Animal House of Osun State University, Osogbo, Nigeria. The animals were kept in a well-ventilated environment at a temperature of 25 ± 2 °C and 55 ± 5 % relative humidity under a 12/12 h light-dark cycle, and provided with standard rat chow and water *ad libitum*. They were randomly assigned to four experimental groups of five rats each: Group 1 (control) received distilled water; Group 2 received 100 mg/kg body weight (b.wt) of aluminium chloride; Group 3 received aluminium chloride plus 50 mg/kg b.wt of rutin, while Group 4 received 50 mg/kg b.wt of rutin only. Treatments were administered using oral gavage for twenty-one days.

### Preparation of serum and tissue homogenates

At the end of the experimental period, blood samples were collected from the orbital venous plexus of the rats into plain bottles. The blood samples were left to clot and were then centrifuged at 4000 rpm for 15 minutes. The serum obtained was refrigerated at -20 °C until required for analysis. The rats were then sacrificed using cervical dislocation and dissected to excise the liver and kidneys. The excised organs were rinsed in ice-cold 1.15% KCl, blotted with filter paper, and weighed. The tissues were then homogenized in 5 volumes of ice-cold 0.1 M phosphate buffer (pH 7.4), using a Teflon homogenizer. The resulting homogenates were centrifuged at 10,000 x g for 20 minutes in a cold centrifuge at 4 °C to obtain the post-mitochondrial fractions. The supernatant was separated and stored in the refrigerator at 4 °C for antioxidant analysis.

### Biochemical analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were analyzed following the protocols of Reitman and Frankel [17]. Gamma-glutamyl transferase (GGT) activity and total bilirubin concentration were assayed using the methods of Schumann et al. (18) and Jendrassik and Grof [19], respectively. Serum creatinine was estimated using Jaffe's picrate method (20). Finally, serum urea levels were estimated using the Berthelot method (21).

Lipid peroxidation in the tissue homogenates was assessed by measuring the formation of thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalents, following the method

of Preuss et al., (22), while nitrate concentration was determined colorimetrically using the Griess reaction (23), and intracellular reactive oxygen species (ROS) formation was evaluated using the oxidation-sensitive dye DCFH (24). Reduced glutathione (GSH) levels were measured according to Beutler et al. (25). Glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities were assayed using modified methods of Matkovics et al. (26) and Habig et al. (27), respectively. Superoxide dismutase (SOD) activity was determined by the procedure of Misra and Fridovich (28), and catalase (CAT) activity was analyzed following the method described by Claiborne [29]. All measurements were performed spectrophotometrically, using a Multiskan FC Microplate Reader Spectrophotometer.

#### Histopathological examination

The liver and kidney tissues were excised and fixed in 10% formalin solution immediately after removal. The specimens were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Serial sections were cut, stained with hematoxylin and eosin, and examined under a light microscope for the evaluation of histopathological changes [30].

#### Statistical analysis

GraphPad Prism (GraphPad Software v. 7.1, USA) was used for statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test, and results were presented as mean ± standard error of the mean (S.E.M.). A priori power analysis was performed, and statistical significance was set at  $p < 0.05$ .

## RESULTS

The body weight changes in the experimental rats are shown in Table 1. One-way ANOVA showed a significantly decreased body weight ( $F(3,16) = 11.29$ ,  $p = 0.0003$ ) in the  $AlCl_3$ -administered experimental animals, with Tukey's post hoc test showing a decline in body weight ( $p = 0.0002$ ,  $q = 7.86$ ;  $df = 16$ ) compared to the control group. However, co-administration of  $AlCl_3$  and rutin significantly improved the body weight gain ( $p = 0.0206$ ,  $q = 4.69$ ).

The serum levels of AST, ALT, GGT, bilirubin, urea, and creatinine are shown in Table 2. One-way ANOVA showed a significant toxicity effect on AST ( $F(3, 16) = 8.33$ ,  $p = 0.0015$ ) and ALT ( $F(3, 16) = 7.65$ ,  $p = 0.0022$ ). Tukey's post hoc test showed that  $AlCl_3$  significantly elevated AST and

ALT compared with the control (AST:  $p = 0.0034$ ,  $q = 5.94$ ; ALT:  $p = 0.0068$ ,  $q = 5.46$ ;  $df = 16$ ), while rutin co-treatment significantly attenuated these increases (AST:  $p = 0.0128$ ,  $q = 5.02$ ; ALT:  $p = 0.0031$ ,  $q = 6.00$ ). GGT and bilirubin were also significantly affected (GGT:  $F(3, 16) = 122.90$ ,  $p < 0.0001$ ; bilirubin:  $F(3, 16) = 44.00$ ,  $p < 0.0001$ ) with  $AlCl_3$  exposure significantly elevating GGT and bilirubin levels (GGT:  $p < 0.0001$ ,  $q = 23.410$ ; bilirubin:  $p < 0.0001$ ,  $q = 15.12$ ;  $df = 16$ ). However, co-treatment with rutin significantly reversed these elevations (GGT:  $p < 0.0001$ ,  $q = 18.80$ ; bilirubin:  $p < 0.0001$ ,  $q = 12.20$ ). Similarly, serum urea and creatinine levels showed significant group differences (urea:  $F(3, 16) = 50.79$ ,  $p < 0.0001$ ; creatinine: ( $F(3,16) = 58.54$ ,  $p < 0.0001$ ) with the administration of  $AlCl_3$ , significantly increasing urea and creatinine concentrations (urea:  $p < 0.0001$ ,  $q = 14.78$ ; creatinine:  $p < 0.0001$ ,  $q = 16.20$ ;  $df = 16$ ). However, co-treatment with rutin significantly mitigated these elevations (urea:  $p < 0.0001$ ,  $q = 15.270$ ; creatinine:  $p < 0.0001$ ,  $q = 10.32$ ).

Antioxidant and lipid peroxidation analyses for the kidney and liver are summarized in Tables 3 and 4, respectively. One-way ANOVA showed that  $AlCl_3$  administration significantly increased MDA (kidney:  $F(3, 16) = 32.11$ ,  $p < 0.0001$ ; liver:  $F(3, 16) = 89.04$ ,  $p < 0.0001$ ) and ROS (kidney:  $F(3, 16) = 46.53$ ,  $p < 0.0001$ ; liver:  $F(3, 16) = 55.99$ ,  $p < 0.0001$ ) compared to the control, with Turkey's post hoc test showing significantly elevated levels of MDA (kidney:  $p < 0.0001$ ,  $q = 12.91$ ; liver:  $p < 0.0001$ ,  $q = 21.56$ ;  $df = 16$ ) and ROS (kidney:  $p < 0.0001$ ,  $q = 14.53$ ; liver:  $p < 0.0001$ ,  $q = 18.26$ ;  $df = 16$ ). However, co-treatment with rutin significantly decreased the MDA (kidney:  $p = 0.47$ ,  $q = 2.10$ ; liver:  $p < 0.0001$ ,  $q = 9.55$ ) and ROS (kidney:  $p = 0.0002$ ,  $q = 8.05$ ; liver:  $p = 0.0003$ ,  $q = 7.72$ ) concentrations toward levels comparable to the control group.

**Table 1.** Body weight changes in Wistar rats exposed to  $AlCl_3$  and rutin

Group	Average initial body weight (g)	Average final body weight (g)	Average weight changes (g)
Control	141.20 ± 8.93	156.80 ± 9.26	+15.60
$AlCl_3$	140.80 ± 6.90	131.80 ± 5.50	-9.00 <sup>a</sup>
Rutin + $AlCl_3$	140.70 ± 4.67	146.20 ± 5.33	+5.50 <sup>a,b</sup>
Rutin only	140.40 ± 7.30	151.00 ± 7.60	+10.60 <sup>b</sup>

Values are expressed as mean ± SEM of 5 replicates; <sup>a</sup> $p < 0.05$  vs control and <sup>b</sup> $p < 0.05$  vs  $AlCl_3$ .

**Table 2.** Kidney and liver function parameters in Wistar rats exposed to AlCl<sub>3</sub> and rutin

Analysis	Control	AlCl <sub>3</sub>	Rutin + AlCl <sub>3</sub>	Rutin only
AST (U/L)	83.48 ± 3.71	104.24 ± 5.69 <sup>a</sup>	78.25 ± 3.22 <sup>b</sup>	72.55 ± 2.34 <sup>b</sup>
ALT (U/L)	74.82 ± 2.80	95.54 ± 1.86 <sup>a</sup>	75.56 ± 4.38 <sup>b</sup>	85.20 ± 3.43
GGT (U/L)	41.74 ± 3.26	184.71 ± 4.27 <sup>a</sup>	75.84 ± 1.12 <sup>a,b</sup>	37.58 ± 4.91 <sup>b</sup>
Bilirubin (µmol/L)	50.47 ± 2.02	72.08 ± 1.03 <sup>a</sup>	54.64 ± 1.49 <sup>b</sup>	62.77 ± 0.92 <sup>a,b</sup>
Urea (mmol/L)	23.92 ± 1.07	39.53 ± 1.39 <sup>a</sup>	23.4 ± 0.78 <sup>b</sup>	27.33 ± 0.89 <sup>b</sup>
Creatinine (mmol/L)	44.60 ± 4.90	131.60 ± 2.64 <sup>a</sup>	82.20 ± 3.58 <sup>a,b</sup>	44.40 ± 5.91 <sup>b</sup>

Values are expressed as mean ± SEM of 5 replicates; <sup>a</sup>p < 0.05 vs control and <sup>b</sup>p < 0.05 vs AlCl<sub>3</sub>

**Table 3.** Kidney antioxidants and lipid peroxidation markers in Wistar rats exposed to AlCl<sub>3</sub> and rutin

Analysis	Control	AlCl <sub>3</sub>	Rutin + AlCl <sub>3</sub>	Rutin only
Catalase (µM/ml/mins)	11.75 ± 0.13	9.94 ± 0.17 <sup>a</sup>	9.53 ± 0.34 <sup>a</sup>	9.85 ± 0.37 <sup>a</sup>
Glutathione (mM)	0.37 ± 0.003	0.27 ± 0.008 <sup>a</sup>	0.45 ± 0.02 <sup>a,b</sup>	0.44 ± 0.03 <sup>a,b</sup>
Glutathione-S-transferase (µM/min/ml)	0.046 ± 0.001	0.02 ± 0.003 <sup>a</sup>	0.04 ± 0.0004 <sup>b</sup>	0.04 ± 0.001 <sup>b</sup>
Glutathione peroxidase (mM)	16.15 ± 0.19	11.68 ± 0.23 <sup>a</sup>	119.07 ± 0.028 <sup>a,b</sup>	20.62 ± 0.22 <sup>a,b</sup>
Superoxide dismutase (U/ml)	0.67 ± 0.08	0.20 ± 0.007 <sup>a</sup>	0.66 ± 0.021 <sup>b</sup>	0.57 ± 0.03 <sup>b</sup>
Nitric oxide (µM)	8.70 ± 0.67	1.06 ± 0.23 <sup>a</sup>	7.12 ± 0.06 <sup>b</sup>	6.56 ± 0.36 <sup>a,b</sup>
Malondialdehyde (µM)	7.59 ± 0.03	10.86 ± 0.3 <sup>a</sup>	8.12 ± 0.36 <sup>b</sup>	9.03 ± 0.19 <sup>a,b</sup>
Reactive oxygen species	0.46 ± 0.002	0.87 ± 0.04 <sup>a</sup>	0.68 ± 0.03 <sup>a,b</sup>	0.48 ± 0.03 <sup>b</sup>

Values are expressed as mean ± SEM of 5 replicates; <sup>a</sup>p < 0.05 vs control and <sup>b</sup>p < 0.05 vs AlCl<sub>3</sub>

**Table 4.** Liver antioxidants and lipid peroxidation marker in Wistar rats exposed to AlCl<sub>3</sub> and rutin

Analysis	Control	AlCl <sub>3</sub>	Rutin + AlCl <sub>3</sub>	Rutin only
Catalase (µM/ml/mins)	7.16 ± 0.21	4.36 ± 0.05 <sup>a</sup>	7.43 ± 0.18 <sup>b</sup>	7.91 ± 0.20 <sup>a,b</sup>
Glutathione (mM)	0.37 ± 0.01	0.24 ± 0.009 <sup>a</sup>	0.33 ± 0.005 <sup>a,b</sup>	0.37 ± 0.004 <sup>b</sup>
Glutathione-S-transferase (µM/min/ml)	0.21 ± 0.008	0.072 ± 0.006 <sup>a</sup>	0.092 ± 0.003 <sup>a</sup>	0.17 ± 0.007 <sup>a,b</sup>
Glutathione peroxidase (mM)	57.91 ± 3.29	33.02 ± 0.82 <sup>a</sup>	66.6 ± 0.59 <sup>b</sup>	110.59 ± 3.00 <sup>a,b</sup>
Superoxide dismutase (U/ml)	1.11 ± 0.04	0.53 ± 0.017 <sup>a</sup>	1.32 ± 0.032 <sup>a,b</sup>	1.22 ± 0.05 <sup>b</sup>
Nitric oxide (µM)	9.64 ± 0.19	3.85 ± 0.16 <sup>a</sup>	9.57 ± 0.22 <sup>b</sup>	10.53 ± 0.06 <sup>a,b</sup>
Malondialdehyde (µM)	7.59 ± 0.18	13.23 ± 0.13 <sup>a</sup>	10.09 ± 0.46 <sup>a,b</sup>	8.55 ± 0.13 <sup>b</sup>
Reactive oxygen species	0.34 ± 0.01	1.14 ± 0.017 <sup>a</sup>	0.68 ± 0.06 <sup>a,b</sup>	0.72 ± 0.07 <sup>a,b</sup>

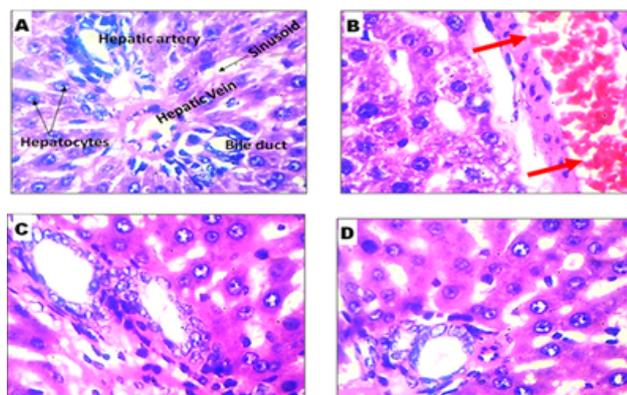
Values are expressed as mean ± SEM of 5 replicates; <sup>a</sup>p < 0.05 vs control and <sup>b</sup>p < 0.05 vs AlCl<sub>3</sub>

The inflammatory biomarker nitric oxide was significantly reduced (kidney: F(3, 16) = 69.24, p < 0.0001; liver: F(3, 16) = 334.00, p < 0.0001) in the AlCl<sub>3</sub>-administrated group as shown by one-way ANOVA and supported by Turkey's post hoc test (kidney: p < 0.0001, q = 19.11; liver: p < 0.0001, q = 34.52; df = 16). Co-treatment with rutin significantly attenuated this effect (kidney: p = 0.0573, q = 3.95; liver: p = 0.9900, q = 0.39) when compared with the control group.

Furthermore, one-way ANOVA showed a significant reduction of SOD (kidney: F(3, 16) = 27.55, p < 0.0001; liver: F(3, 16) = 101.80, p < 0.0001) and catalase (kidney: F(3, 16) = 13.46, p = 0.0001; liver: F(3, 16) = 86.70, p < 0.0001) activities in the liver and kidney following AlCl<sub>3</sub>-exposure with Turkey's post hoc test showing significantly decreased SOD (kidney: p < 0.0001, q = 11.19; liver: p < 0.0001, q = 16.51; df = 16) and catalase (kidney: p = 0.0013, q = 6.62; liver: p < 0.0001, q = 16.29; df = 16) activities. These activities were significantly increased following co-

treatment with rutin for SOD (kidney:  $p = 0.9947$ ,  $q = 0.35$ ; liver:  $p = 0.0034$ ,  $q = 5.95$ ) and catalase (kidney:  $p = 0.0002$ ,  $q = 8.105$ ; liver:  $p = 0.6921$ ,  $q = 1.562$ ) to levels indistinguishable from the control group.

Also, one-way ANOVA of GPx (kidney:  $F(3, 16) = 453.20$ ,  $p < 0.0001$ ; liver:  $F(3,16) = 97.19$ ,  $p < 0.0001$ ) and GST (kidney:  $F(3, 16) = 64.80$ ,  $p < 0.0001$ ; liver:  $F(3, 16) = 44.50$ ,  $p < 0.0001$ ) activities, together with GSH (kidney:  $F(3, 16) = 26.43$ ,  $p < 0.0001$ ; liver:  $F(3, 16) = 70.52$ ,  $p < 0.0001$ ) concentration, revealed significant group differences. Administration of  $AlCl_3$  significantly increased GPx (kidney:  $p < 0.0001$ ,  $q = 24.22$ ; liver:  $p = 0.0006$ ,  $q = 7.18$ ;  $df = 16$ ) and GST (kidney:  $p < 0.0001$ ,  $q = 17.88$ ; liver:  $p < 0.0001$ ,  $q = 13.87$ ;  $df = 16$ ) with a concomitant decrease in the GSH concentration (kidney:  $p = 0.0020$ ,  $q = 6.32$ ; liver:  $p < 0.0001$ ,  $q = 18.21$ ;  $df = 16$ ). However, co-treatment with rutin significantly mitigated GPx (kidney:  $p < 0.0001$ ,  $q = 15.78$ ; liver:  $p = 0.3215$ ,  $q = 2.51$ ) and GST (kidney:  $p = 0.1960$ ,  $q = 2.97$ ; liver:  $p < 0.0001$ ,  $q = 11.71$ ) elevations and GSH reduction (kidney:  $p = 0.0157$ ,  $q = 4.88$ ; liver:  $p = 0.0017$ ,  $q = 6.44$ ) to levels comparable to the control group.

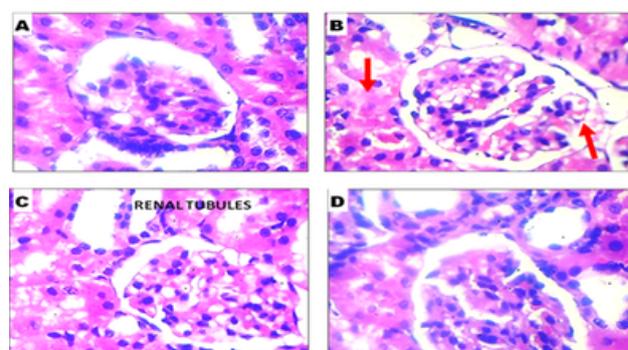


**Figure 1.** Photomicrographs of hematoxylin and eosin (H&E)-stained hepatic cells ( $\times 400$ ) from rats treated with  $AlCl_3$  and rutin

A: Control group showed a well-outlined cellular profile with no altered panoramic morphological presentation, with hepatocytes well distributed across the general cytoarchitecture. B: Animals treated with  $AlCl_3$  only showed derangement in cellular profiles characterized by severe loss of liver parenchyma, severe hemorrhage, infiltration of inflammatory cells within and around the central vein and sinusoids, and dilatation of the hepatic portal triad. (red thick arrows). C: Animals co-administered  $AlCl_3$  and rutin showed a mild alteration in cytoarchitecture, less conspicuous than seen in B. D: Animals treated with rutin only also showed a well outlined cellular profile and well distributed hepatocytes across the general cytoarchitecture.

Figure 1 reveals the liver histopathological examination using H and E staining.  $AlCl_3$ -exposed animals showed cellular profile derangement characterized by severe loss of liver parenchyma, severe hemorrhage, and infiltration of inflammatory cells within and around the central vein and sinusoids, and dilatation of the hepatic portal triad. Co-administration of rutin in  $AlCl_3$ -exposed rats showed mild alterations in cytoarchitecture, revealing the protective effect of rutin.

Similarly, renal tissue histology showed preserved renal architecture and normal renal corpuscles in control rats, severe focal sclerosis of the glomerulus, hemorrhagic changes, and observable red inflammatory cells in  $AlCl_3$ -exposed rats. The concomitant administration of rutin and  $AlCl_3$  preserved renal histoarchitecture with mild fibrosis and hemorrhage, characterized by the presence of red inflammatory cells, as shown in Figure 2.



**Figure 2.** Photomicrographs of hematoxylin and eosin (H&E)-stained kidney cells ( $\times 400$ ) from rats administered  $AlCl_3$  and rutin

A: Control group showed no observable degenerative changes, and well-arranged renal corpuscles, glomeruli, macula densa, and convoluted tubules. B: Animals treated with  $AlCl_3$  only showed severe focal sclerosis of the glomerulus with hemorrhagic changes, together with observable red inflammatory cells. C: Animals co-administered  $AlCl_3$  and rutin showed mild fibrosis and hemorrhage characterized by the presence of red inflammatory cells, with no other varying degrees of renal injury. D: Animals treated with rutin showed no observable degenerative changes, while the renal corpuscles, glomeruli, macula densa, and convoluted tubules were well arranged.

## DISCUSSION

Previous research has shown that weight gain is a reliable marker for assessing substance toxicity (31). A significant decrease in body weight gain in Al-treated rats, as seen in this study, affirms this notion. This decrease may be due to depletion of body fluids and adipose tissue, or reduction of food intake, leading to nutritional malabsorption (32). Our findings thus provide evidence of Al toxicity, as it impairs body weight gain. However, rutin, a naturally occurring flavonoid glycoside, protects cells against harmful effects, including free radicals, due to its antioxidant, anti-inflammatory, and antiapoptotic properties (33). This enhances rutin's potential to support body weight gain in Al-exposed rats, stimulating anabolic processes and suppressing tissue breakdown.

Organ injury causes membrane damage or necrosis. As demonstrated by our findings, a significant elevation of hepatic enzymes (AST and ALT), GGT, and bilirubin concentration in the serum of Al-exposed rats indicates liver dysfunction (34). This is caused by changes in the permeability of hepatocyte membranes resulting from free radical damage, inflammation, and cellular injury (35). Rutin administration significantly lowered the hepatotoxic effects of Al, as evidenced by reductions in the serum AST, ALT, GGT, and bilirubin, supporting rutin hepatoprotective ability as reported in previous studies (36,37). The current investigation also discovered a significant increase in urea and creatinine levels in Al-exposed rats. This shows the kidney's inability to effectively filter the metabolic waste products, suggesting extensive disruption of renal structure and function (38). The administration of rutin restored urea and creatinine concentration to levels similar to those of the control group, which agrees with the findings of Kucukler et al. (39).

ROS serve as signaling molecules that regulate and maintain homeostasis at physiological concentrations, including hepatorenal homeostasis. Excessive generation of ROS causes oxidative stress by triggering phospholipid peroxidation (LPO), impairing membrane and organelle functions, and eventually leading to death (40). In this study, elevated ROS and MDA concentrations, as well as a reduction in nitric oxide concentration following  $AlCl_3$  induction, agree with previous studies [41, 42]. This suggests that Al induces hepatorenal toxicity by enhancing free radical-mediated tissue and cellular damage and potentiates iron-mediated lipid peroxidation (43, 44). Rutin administration improved oxidative stress by stabilizing ROS, MDA, and nitric oxide concentrations to

levels close to those of the control group, thereby preventing Al hepatorenal toxicity. This result is plausible as rutin acts as an ROS scavenger by donating hydrogen atoms to superoxide anions and hydroxyl and peroxy radicals (45). It is also a master redox switch through Nrf2 activation and iNOS upregulation (46, 47).

Besides accompanying MDA accumulation as discussed, the present study also observed reduced GSH as well as repressed activities of SOD, catalase, GST, and GPx in the hepatic and renal tissues of Al-exposed animals. The roles of these antioxidant biomarkers in maintaining tissue redox-homeostasis cannot be overemphasized. SOD converts superoxide radicals into hydroxyl peroxide, which is further converted by catalase into water [48, 49]. GPx oxidizes GSH into GSSG, which is then reduced to GSH by glutathione reductase [49]. The decline in the levels of these antioxidants following Al-exposure suggested they were overwhelmed by Al-induced oxidative stress.

Although a reduced renal catalase antioxidant activity was observed, rutin was effective in reversing the Al-toxicity effects on both renal and hepatic tissues by increasing GSH concentration and enhancing SOD, catalase, GST, and GPx activities. This finding aligns with previous studies, suggesting rutin's ability to directly scavenge the generated ROS, such as hydroxyl radicals,  $H_2O_2$ , and superoxide anions, thereby maintaining the body's redox balance [50].

The histological examination of kidney and liver sections revealed distorted cytoarchitecture and cellular degeneration in Al-exposed rats, which agrees with the findings of Samir et al. (42). These alterations might be due to severe cell membrane damage, increased permeability of the plasma membrane, and vascular alteration-induced hemorrhage [51]. Rutin administration exhibited protective effects on the kidney and liver, thereby improving the distorted cytoarchitecture and cellular degeneration observed in Al-exposed rats. This can be attributed to the antioxidant potency of rutin, which significantly reduced the oxidative stress and cell damage.

This study establishes the therapeutic potential of rutin against Al-induced toxicity, as it attenuates functional damage in both the kidney and liver tissues. It also serves as a promising antioxidant and anti-inflammatory agent in alleviating oxidative stress and tissue inflammation posed by heavy metals, especially aluminium. This observation suggests that rutin could be recommended as a daily dose for prevention, particularly for individuals at high risk of aluminium exposure. However, the therapeutic use of rutin for the management of hepatorenal damage should be

used with caution, given its potential adverse effects in other clinical conditions.

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### Author's Contribution

Conceptualization, O.I.O.; Methodology, O.I.O.; Supervision, O.I.O.; Investigation, A.M.B.; Writing – original draft, A.M.B.; Writing – review & editing, O.I.O. Both authors have read and approved the published version of the manuscript.

### Statement of Ethics

This study was conducted in accordance with the ethical guidelines for the care and use of laboratory animals. The protocol was approved under the approval number UNIOSUNHREC 2026/002B by the Health Research Ethics Committee (HREC) of Osun State University, Osogbo, Nigeria.

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### Statement of Competing Interest

The authors declare no relevant conflicts of interest.

### Statement of Data Availability

All data analyzed during this study are included within the published article.

### Statement of Generative AI Technologies Use

No generative AI was used.

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