Journal of Research in Applied and Basic Medical Sciences

ORIGINAL RESEARCH ARTICLE

Vitamin E Ameliorates Sodium Fluoride-Induced Morphometric, Histomorphological, and Biochemical Changes on the Kidney of Adult Wistar Rat

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Published: 15 August 2025

Abstract

Background Sodium fluoride, NaF, usage in dental products and drinking water is one of the most promising methods of dental caries management; however, its toxicity generates free radicals. Vitamin E is a recognized antioxidant that helps protect against tissue damage. The study was aimed at investigating whether vitamin E at the tolerable upper intake level could ameliorate NaF-induced toxicity on kidney morphometry, histomorphology, and serum biochemical markers of renal function in adult male Wistar rats.

Methods A total of 30 male Wistar rats weighing 130-180 g were randomly divided into six groups of five animals each. Group A and B served as the control and received 1 ml of distilled water and 1 ml of Tween 80®; Group C received 5 mg/kg body weight of NaF, Group D received 20 mg/kg body weight of NaF while Group E and F received 5 mg/kg and 20 mg/kg and 14.3 mg/day of vitamin E orally, respectively.

Results After 45 days, morphometry of the kidney showed a significant ($p < 0.05$) increase in length, thickness, and width of the inferior pole in NaF groups only, but significantly decreased in Group E compared with the control. Histomorphology showed various changes in Groups C and D, while Groups E and F showed mild modifications compared to the control. Masson's trichrome stain showed pale-stained collagen in NaF groups (C and D) but increased staining in vitamin E groups (E and F). Serum creatinine and urea levels were significantly increased in Group D but decreased in Groups E and F. Electrolytes (Na^+, Cl^-, and $HCO3^-$) were significantly increased in the NaF-treated groups but decreased with vitamin E compared to the control.

Conclusion NaF induced significant changes in kidney morphometry, histomorphology, and serum biochemical markers of renal function. Vitamin E administration at the upper tolerable intake level can mitigate the changes.

Keywords Histomorphology, Kidney, Morphometry, Renal function, Sodium fluoride, Vitamin E

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Received: 01 May 2025/ Revised: 13 August 2025/ Accepted: 28 August 2025

1 Introduction

Over the years, sodium fluoride (NaF) has emerged as a compound of significant public health concern globally due to its widespread nature in the environment. Several studies showed a thin line between its benefits and toxicity, making NaF a two-edged sword on exposure. The World Health Organization (WHO) recommended a limit of 1.5 ppm for fluoride in drinking water, while the Bureau of Indian Standards recommends even a lower range of 0.6-1.2 ppm[1] in fluoride endemic areas. In Nigeria, a Sub-Saharan country, a study by Ogbu et al.[2] indicated that underground water sources are prone to elevated NaF concentrations. Due to the country's inadequate water supply systems, over 80% of the population is forced to rely on underground water sources, such as wells, boreholes, and streams, for drinking and domestic purposes. In certain areas, the NaF concentrations from the aforementioned water sources range from 0.2 - 8 mg/L, surpassing the WHO's recommended maximum value of 1.5 mg/L.[3] This situation poses significant risks to public health, with reports indicating chronic glomerulonephritis as one of the leading causes of end-stage renal disease (ESRD) in Nigeria[4]

Chronic kidney disease (CKD) is currently emerging as a worldwide public health problem with an estimated 200 million people in the low and middle-income countries of Asia and Sub-Saharan Africa reportedly said to have the highest rates of CKD globally[4] In Nigeria, CKD is said to account for about 8–10% of hospital admissions, and because it is well known that CKD is underreported, patients with end-stage renal disease are thought to represent the tip of the iceberg of the entire burden of CKD. Previous studies had linked age, elevated blood pressure, diabetes mellitus, habitual intake of analgesics, herbs, obesity,[5] and glomerulonephritis[4] as threats. However, non-traditional threats such as oxidative stress and inflammation are said to be more prevalent in patients with CKD than in normal subjects.[6] Since environmental toxins have been linked to CKD in people living in fluoride endemic areas.[7-9] Consequently, there is a pressing need for more in-depth studies on fluoride toxicity, particularly its impact on kidney morphometry, histomorphology, and function, focusing on areas where underground NaF levels exceed the recommended limit. Given that oxidative stress has been established as a mechanism of action of NaF-induced toxicity,[10] antioxidant intervention could mitigate such effects. In this regard, current research interest has been drawn to the potential of antioxidants from plants in mitigating oxidative stress and inflammation on renal function and morphology.

Vitamin E, a fat-soluble vitamin synthesized by plants from tyrosine-derived quinones,[11] is a known antioxidant that could mitigate NaF-induced renal damage by counteracting oxidative stress, thereby offering protective benefits in fluoride-endemic regions. Among the various forms of vitamin E, α-tocopherol is recognized as the most biologically active form of vitamin E. This particular form is the potent form which acts as a peroxyl radical scavenger, inhibiting the generation of harmful free radicals within tissues[12] Vitamin E is primarily located in cell membranes, where it plays a crucial role in protecting cells from oxidative damage.[13]

Therefore, the objective of our study was to investigate whether the administration of vitamin E at the daily tolerable upper intake level (UL) could ameliorate NaF toxicity on kidney morphometry, histomorphology, and serum biochemical markers of renal damage in adult male Wistar rats.

2 Methods

Animals

Adult male Wistar rats were obtained from the Animal House Unit of the College of Health Sciences, University of Uyo, in Uyo, Nigeria. The animals were allowed to acclimatize for two weeks before administration. After acclimatization, the animals were randomly divided into six groups of five animals each. The groups were labeled A, B, C, D, E, and F, and they were administered their respective dosages. The animals were housed in wooden cages with stainless steel grill tops and were maintained under room temperature and hygienic conditions. They were exposed to a 12-hour light/dark cycle. The animals were maintained on standard pelletized feeds (Vital Feeds, by GCOML Ltd.) and tap water. Administration was done once per day in the morning (7-8 a.m.) orally using an oral cannula for 45 days, and animals were weighed weekly and observed daily throughout the period.

Experimental Design

Thirty male Wistar rats, with body weights ranging from 130 g to 180 g, were randomly allocated into six groups, each comprising five animals, as shown in Table 1. Vitamin E was administered about 5-10 minutes after NaF administration. All treatment was done orally.

Table 1 Experimental design

Group	Treatment	Dose
A	Distilled water (control)	1 ml
B	Tween 80® (vehicle control)	1 ml
C	Sodium fluoride (NaF)	5 mg/kg body weight
D	Sodium fluoride (NaF)	20 mg/kg body weight
E	NaF + vitamin E	5 mg/kg body weight NaF + 14.3 mg/day vitamin E
F	NaF + vitamin E	20 mg/kg body weight NaF + 14.3 mg/day vitamin E

Chemicals

NaF with the CAS No. [7681-49-4] of 98% purity from Guangdong Guanghua Sci-Tech Co., Ltd, and Vi Boost (Vitamin E capsules USP 1000 IU) from Softgel Healthcare Private Ltd, Tamilnadu, with manufacturing license No. TN00002124, as well as Tween 80® from Sigma-Aldrich, were used in this study.

The doses of NaF administered were prepared from the median lethal dose (LD50) value of 52 mg/kg body weight for rats administered orally.[14] The doses were administered such that each animal received approximately 10% and 40% of the LD50 of NaF. In addition, the doses used in the present study were in agreement with those in previous studies ,[15-17] aiming to prevent fatal damage to the animals. Whereas, vitamin E used was based on the U.S. Institute of Medicine tolerable upper ULs of 1000 mg/day.[18] The 10% of the dosage used represented a low dose of the LD50, while 40% served as the high dose of the LD50. The dosage was tripled to evaluate whether at high toxicity, vitamin E at the tolerable upper UL could still mitigate the changes.

Preparation of NaF

To achieve a concentration of 1 mg/1 ml, 500 mg of NaF was dissolved in 500 ml of distilled water and served as the stock solution. A concentration of 50 mg/50 mL was used per day, serving as the working solution, and was dissolved in distilled water and stirred to ensure thorough mixing.

Preparation of Vitamin E

A volume of 70 ml of distilled water was warmed in a beaker, while 30 ml of Tween 80® was added to it and mixed using a glass stirrer to make 30% of Tween 80®. A vitamin E capsule was cut and diluted in 30% of Tween 80®. This was further stirred to ensure it was dissolved.

Termination of Experiment

The animals were sacrificed by chloroform inhalation on day 46. The thoraco-abdominal wall was then dissected to access the heart, and blood was aspirated from the left ventricle of the heart using a 5 ml syringe into plain bottles and immediately placed in ice blocks. The blood was then centrifuged at 3,500 rpm for 15 minutes. The serum was collected in new plain sample bottles and refrigerated for serum biochemical analyses. Kidneys were dissected out and weighed on a weighing balance (Metler Electric Balance Model: MT-301) and washed in standard saline solution. The kidneys were then fixed in 10% buffered formalin for 48 hours for histological and histochemical analyses.

Morphometric Analysis

The kidney dimensions, length, width at superior and inferior poles, as well as thickness, were measured using a digital vernier caliper (EIE Instrument PVT Ltd.) according to Murlimanju et al.[19] Organosomatic indices (OSI) of the kidneys were determined as used by Adesina.[20]

$$\frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100\%$$

Biochemical Analyses

Determination of Creatinine

Serum creatinine level was determined by Jaffe's reaction as described by Slot.[21] Randox reagents kits (RX MONZA CR 510: Randox Laboratory Ltd., United Kingdom) were used. Sample serum creatinine concentration (µmol/L) was calculated using the formula by Slot:[20]

$$\frac{(\Delta \text{ sample})}{(\Delta \text{ standard})} \times \text{Standard concentration } (\mu\text{mol/L})$$

Where Δ sample = absorbance of the sample = $A_2 - A_1$

Δ standard = absorbance of the standard against the blank (A2 of standard - A1 of standard).

Determination of Urea

Urea was determined based on the principle of Berthelot's reaction as described by Fawcett and Scott,[22] using the formula below:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Determination of Electrolytes

All electrolyte concentrations were determined using Randox reagent kits, following the procedures provided by the manufacturer. Measurement of the electrolytes sodium and potassium was conducted using the ion-selective electrode method, originally described by Fogh-Andersen et al.[23] Chloride levels were determined using the ion-selective electrode method as outlined by Guagnellini et al.[24] Bicarbonate was measured through the back titration method according to Boone and Field.[25]

Tissue Processing

Tissue sections of the kidney were processed using the paraffin embedding method, sectioned at 5 µm, and stained with the routine haematoxylin and eosin (H&E) stain, as described by Bancroft et al.[26]

Masson's trichrome (MT) staining technique was used according to Prophet et al.[27] for formalin-fixed paraffin-embedded tissue sectioned at 5 µm.

Statistical Analysis

Statistical analyses were carried out using the statistical analysis package (IBM SPSS software version 20). One-way analysis of variance (ANOVA) and post-hoc (Tukey's

multiple comparison tests) were used to determine if there was any significance between the groups compared with the control. All data were expressed as Mean ± Standard Error of the Mean (SEM), and tables were used to illustrate the variations in numerical values across experimental groups. A p-value of < 0.05 was considered to be statistically significant.

This study was a random qualitative analysis; therefore, quantification of the tissue damage and measurement of the intensity of the collagen stain were not carried out. These could be areas for further study.

3 Results

Figure 1 shows the mean body weight changes in the animals 45 days after treatment. Data are presented as the Mean.

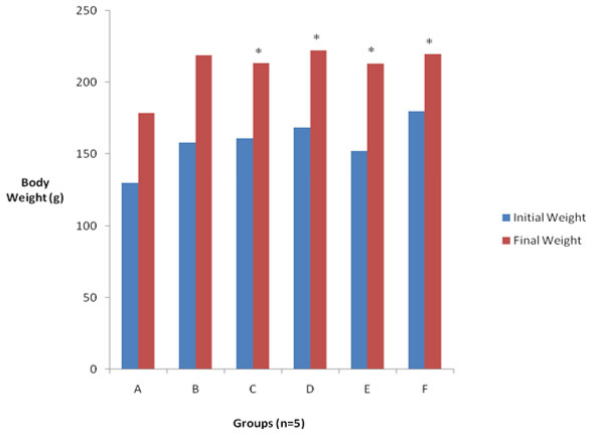

Figure 1 Mean changes in body weight (g) of animals treated with NaF and vitamin E compared with the control

*Significantly different from the initial weight at ($p < 0.05$); A = 1 ml of distilled water; B = 1 ml of Tween 80®; C = 5 mg/kg body weight of NaF; D = 20 mg/kg body weight of NaF; E = 5 mg/kg of NaF + 14.3 mg/day of vitamin E; F = 20 mg/kg of NaF +14.3 mg/day of vitamin E

Table 2 shows the organo-somatic index of the animals. This is important as experimental treatment could affect soft organs, resulting in organomegaly or atrophy.

Table 2 Reno-somatic indices (%) of animals treated with NaF and vitamin E

Group (n = 5)	Reno-somatic (%)
A	0.566 ± 0.0317
B	0.536 ± 0.0150
C	0.540 ± 0.0095
D	0.562 ± 0.0206
E	0.576 ± 0.0314
F	0.572 ± 0.0239

Data are presented as Mean ± SEM; ($p > 0.05$)

Table 3 and Table 4 show the morphometric parameters of the kidney 45 days after experimental treatment. This is important as toxins could have an effect on soft organs' morphometry, thereby affecting the measurement during medical imaging.

Table 5 shows serum creatinine and urea concentrations, which are by-products of metabolism (from muscle and from protein breakdown down respectively) in the animal treated with NaF and vitamin E for 45 days. NaF toxicity produces histopathological damage to the kidney. If the kidney fails to excrete these by-products, it causes their build-up in the bloodstream, hence worsening CKD.

Table 6 shows serum electrolyte concentrations in the animals treated with NaF and vitamin E for 45 days. These electrolytes are maintained at a certain level within the body for normal body function. Elevated electrolyte levels in the bloodstream indicate kidney failure to effectively excrete them.

Histomorphological Observation (H&E)

The photomicrograph of the kidney section of Group A animals revealed a normal cytoarchitecture of the renal cortex. The glomerulus (GM) appeared intact within the Bowman's capsule (BC), with a clear Bowman's space (BS). The proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) were well defined, lined by healthy epithelial cells (EPC). The interlobular blood vessels (ILV) were patent, while juxtaglomerular cells (JGC) were visible at the vascular pole of the glomerulus. No evidence of degeneration, necrosis, or distortion was observed after 45 days (Figure 2A).

The photomicrograph of the kidney section of Group B animals showed normal cytoarchitecture of the renal cortex (Figure 2B).

The sections of the kidney from groups C and D animals revealed a severely distorted cytoarchitecture of the renal cortex. The GM were markedly degenerated (DGM) and exhibited glomerular derangement (GMD) with a pronounced narrowing of BS. Features of atrophy (A), loss of architectural details (LAD) surrounding the tubules, and sloughing of EPCs into the tubular lumina were evident. The PCTs and DCTs displayed prominent dilation, while foci of hyalinization (H) were also observed. The ILVs were present but appeared distorted when compared to the normal control architecture (Figure 3C and Figure 3D).

The sections of the kidney from groups E and F animals revealed a mildly distorted cytoarchitecture of the renal cortex. The GM showed degeneration and glomerular atrophy (GA), with narrowed BS. There is a mild loss of cytoarchitectural details of the JGC, alongside areas of increased cellularity (C) and evidence of tubular cell degeneration (TCB). Despite these changes, the PCTs appeared largely normal, while the DCTs and ILV were preserved. These alterations were less severe compared to groups C and D due to vitamin E at 14.3 mg/day for 45 days (Figure 3E and Figure 3F).

Table 3 Length and thickness of the kidneys of animals treated with NaF and vitamin E

Groups (n = 5)	LRK (mm)	LLK (mm)	TRK (mm)	TLK (mm)
A	14.440 ± 0.353	14.480 ± 0.339	6.794 ± 0.321	6.396 ± 0.396
B	14.896 ± 0.231	15.042 ± 0.324	6.728 ± 0.237	7.492 ± 0.286
C	15.256 ± 0.362	15.086 ± 0.187	7.082 ± 0.149	7.220 ± 0.087*c
D	15.376 ± 0.168	15.926 ± 0.213*	7.304± 0.365*b	7.036 ± 0.431
E	15.408 ± 0.296	15.312 ± 0.338	7.182 ± 0.014	7.284 ± 0.136*d
F	15.250 ± 0.129	15.720 ± 0.212*a	7.196 ± 0.140	7.326 ± 0.228*e

Data are presented as Mean ± SEM; ($p > 0.05$); * Significantly different from the control groups A and B at ($p < 0.05$) *a Significantly different from Group E at ($p < 0.05$), *b Significantly different from the control groups A and B and the treatment Group E and F ($p < 0.05$) *c Significantly different from Group A ($p < 0.05$) *d Significantly different from Group A ($p < 0.05$) *e Significantly different from Group D ($p < 0.05$).

LRK: Length of the right kidney; LLK: Length of the left kidney; TRK: Thickness of the right kidney; TLK: Thickness of the left kidney.

Table 4 Width of the kidneys of animals treated with NaF and vitamin E

Groups (n = 5)	LRK (mm)	LLK (mm)	TRK (mm)	TLK (mm)
A	14.440 ± 0.353	14.480 ± 0.339	6.794 ± 0.321	6.396 ± 0.396
B	14.896 ± 0.231	15.042 ± 0.324	6.728 ± 0.237	7.492 ± 0.286
C	15.256 ± 0.362	15.086 ± 0.187	7.082 ± 0.149	7.220 ± 0.087*c
D	15.376 ± 0.168	15.926 ± 0.213*	7.304± 0.365*b	7.036 ± 0.431
E	15.408 ± 0.296	15.312 ± 0.338	7.182 ± 0.014	7.284 ± 0.136*d
F	15.250 ± 0.129	15.720 ± 0.212*a	7.196 ± 0.140	7.326 ± 0.228*e

Data are presented as Mean ± SEM; ($p > 0.05$); WRSP: Width of the right kidney superior pole; WLSP: Width of the left kidney superior pole; WRIP: Width of the right kidney inferior pole; WLIP: Width The left kidney inferior pole; * Significantly different from the control Group A at ($p < 0.05$); *a Significantly different from Group D ($p < 0.05$)

Table 5 Serum creatinine and urea concentration in animals treated with NaF and vitamin E

Group (n = 5)	Creatinine (μmol/L)	Urea (μmol/L)
A	45.20 ± 2.67	3.64 ± 0.33
B	45.40 ± 3.31	4.00 ± 0.15
C	49.40 ± 3.77	4.06 ± 0.31
D	50.00 ± 5.55*a	4.66 ± 0.44*c
E	47.40 ± 4.55	3.80 ± 0.28
F	48.00 ± 6.47*b	3.80 ± 0.22

Data are presented as Mean ± SEM ($p > 0.05$); *a Significantly different from the control Group A and B at ($p < 0.05$); *b Significantly different from the control Group B at ($p < 0.05$); *c Significantly different from groups E and F at ($p < 0.05$)

Table 6 Serum electrolyte concentrations in animals treated with NaF and vitamin E in mmol/L

Groups (n = 5)	Na^+	K^+	Cl^-	HCO_3^-
A	136.94 ± 2.60	6.88 ± 0.16	96.44 ± 1.99	14.00 ± 0.71
B	134.14 ± 1.22	6.84 ± 0. 23	98.22 ± 0.99	11.00 ± 1.41
C	138.80 ± 1.43a	7.78 ± 0.38	101.26 ± 1.44c	11.00 ± 0.55e
D	139.30 ± 0.99b	7.66 ± 0.34	99.98 ± 1.17	18.40 ± 1.81f
E	135.36 ± 2.45	7.76 ± 0.22	97.58 ± 1.70	15.00 ± 2.30
F	135.32 ± 0.60	7.64 ± 0.11	96.90 ± 0.48d	12.00 ± 0.71

Data are presented as Mean ± SEM; ($p > 0.05$); a Significantly different from the control Group B at ($p < 0.05$); b Significantly different from the groups E and F at ($p < 0.05$); c Significantly different from treatment Group F at ($p < 0.05$); d Significantly different from Group C at ($p < 0.05$); e Significantly different from control Group D at ($p < 0.05$); f Significantly different from groups B and C at ($p < 0.05$).

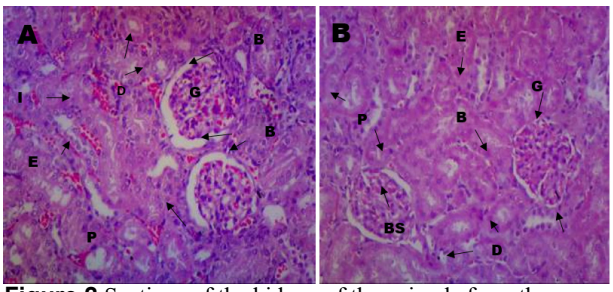

Figure 2 Sections of the kidney of the animals from the control Group A (1 ml distilled water) compared with Group B (1 ml of Tween 80®) (H&E) A, B: ×400

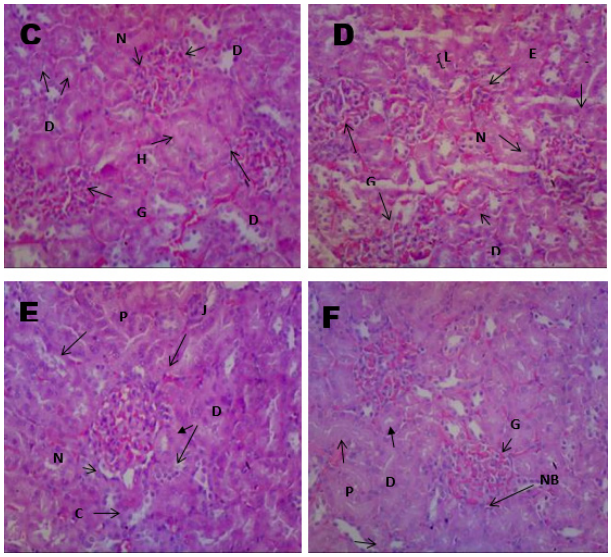

Figure 3 Comparing the sections of the kidney of the animals from Group C (5 mg/kg body wight, NaF only); Group D (20 mg/kg body weight, NaF only); Group E (5 mg/kg body wight, NaF + 14.3 mg/day vitamin E); Group F (20 mg/kg body weight NaF + 14.3 mg/day vitamin E) (H&E) C, D, E, and F: ×400

The photomicrographs of kidney sections from groups A and B animals showed a normal cytoarchitecture of the renal cortex. Collagen (C) was stained blue around the glomeruli (GM) and tubular structures, including the PCTs and DCTs. EPCs and BS were well preserved, while smooth muscle fibers (M) around the blood vessels were stained red (Figure 4A and Figure 4B).

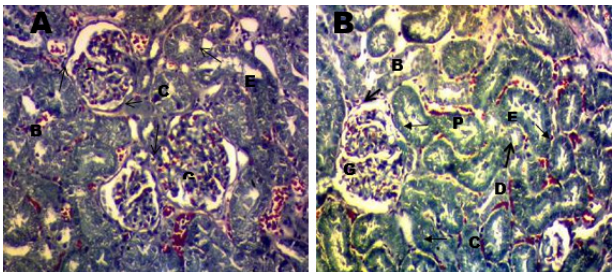

Figure 4 Sections of the kidney of the animals from the control Group A (1 ml distilled water) compared with Group B (1 ml of Tween 80®) (MT) A1, B1: ×400

The photomicrographs of the kidney sections from groups C and D animals showed severe alterations in the cytoarchitecture of the renal cortex. Collagen (C) appeared pale-stained along the tubules, while the glomeruli were necrotic with features of glomerular sclerosis (GS). BS was markedly narrowed, and invagination of the tubules by EPCs was evident. Vacuolation (V) was observed around the podocytes, and smooth muscle fibers (M) surrounding the blood vessels were stained red, in contrast to the normal architecture seen in the control groups (Figure 5C and Figure 5D).

The photomicrographs of the sections of the kidney from groups E and F animals showed mild alterations in the cytoarchitecture at the corticomedullary junction. Collagen (C) deposition was increased around the GM and BS, as well as around the renal tubules. NG were evident, along with loosely attached tubular cells (LTC) and invagination of the tubular lumen by swollen EPCs. These changes were mild compared to the control group (Figure 5E and Figure 5F).

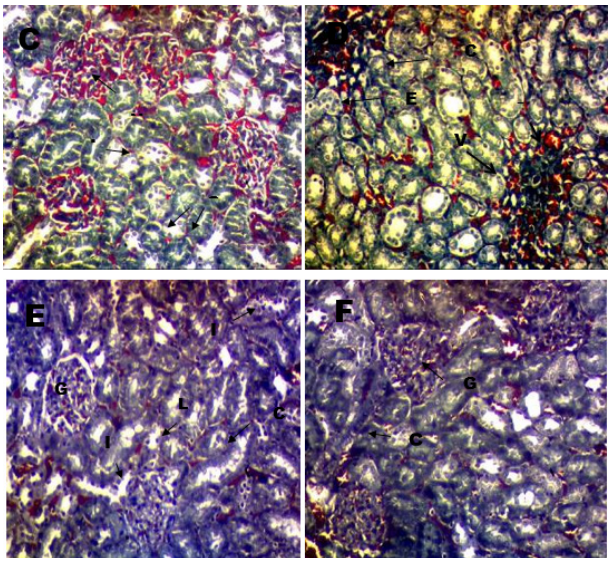

Figure 5 Comparing sections of the kidney of the treated animals from Group C (5 mg/kg body weight, NaF only); Group D (20 mg/kg body weight, NaF only); Group E (5 mg/kg body weight, NaF + 14.3 mg/day vitamin E); Group F (20 mg/kg body weight, NaF + 14.3 mg/day vitamin E) (MT) C1, D1, E1 and F1: ×400

4 Discussion

The final body weight showed a significant increase in all the treatment groups (C-F) ($p < 0.05$) compared to the control Group A, suggesting that NaF toxicity can significantly affect body weight by interfering with metabolic processes and probably favoring anabolism in Wistar rats when vitamin E is supplemented. Also, the increase in body weight observed is supported by the fact

that the animals were well fed. The study is in contrast with previous research by Dhurvey and Thakare[28] that NaF significantly caused a decrease in the body weight of rats at 20 mg/kg/day for 30 days. However, such variations could be attributed to differences in sex, dosage administered, animal age, and study duration. The organs' weights (Table 2) of all the treatment groups (C-F) were not significantly different from the control Groups, implying the treated animals had their normal energy requirement and is consistent with work by Sharma et al.[29] who reported no statistical significance in organs of Swiss albino mice and their organosomatic index treated with NaF, and also with the work of Dhurvey and Thakare[27] that NaF had no significant difference on the left and right ovarian weights at 5 and 10 mg of NaF/kg body weight per day. The renal-somatic indices showed that NaF can affect the reno-somatic index, as evident in the decrease in the renal-somatic index of Group C animals, which were administered NaF, although the decline was not significant. This corroborates a similar report where there was a substantial decrease in the OSI of the kidney in fluoride alone-treated rats, while the treatment of fluoride along with maize purple plant pigment significantly attenuated the reduction induced by fluoride in the OSI of the kidney, indicating the action of an antioxidant.[30]

Morphometric parameters are of clinical significance as they help to determine anatomical variations. Organs' morphometry can also be vital, as it can help to distinguish pathological organs from normal ones (e.g., to distinguish renomegaly from the normal). Its knowledge is thus clinically relevant to surgeons and radiologists. The morphometric parameters of this study showed that the length of the left kidney (LLK) in groups D and F was significantly increased ($p < 0.05$), differing from the control Group A (Table 3). The mean thickness of the right kidney (Table 3) was significantly increased in Group D, which received 20 mg/kg of NaF, while the mean thickness of the left kidney was considerably increased in groups E and F compared with the control Group A. The mean width of the inferior pole of the right kidney (Table 4) decreased significantly in Group E, which received vitamin E supplements, but increased in Group D compared to the control. The significant differences observed could be associated with NaF toxicity exposure, especially at high dosage, while the significant decrease is most likely due to co-administration of vitamin E, contrasting with the findings of Onyeanusi et al.[31] where NaF was not administered.

Creatinine is a vital indicator of renal function, synthesized primarily in the liver through the methylation of glycocyamine (guanidino acetate), which is produced in the kidney from the amino acids' arginine and glycine. It is then transported via the bloodstream and eliminated predominantly by the kidneys through glomerular filtration and proximal tubular secretion.[32]

The significant increase in creatinine concentration observed in Group D animals (Table 4 and Table 5), which were administered 20 mg/kg NaF, was corroborated by histological sections of their kidneys, showing notable histopathological changes. These pathological alterations could be responsible for the elevated serum creatinine levels, as glomerular filtration may be impaired due to degeneration or atrophy resulting from NaF exposure. In contrast, groups E and F, which received vitamin E, showed decreased creatinine concentrations, with the reduction being statistically significant in Group F. This suggests the ameliorative effect of vitamin E against oxidative stress, which can lead to kidney damage and contribute to the development of chronic kidney disease (CKD). These findings are consistent with those of Emejulu et al.[33] who reported that NaF administration significantly increased serum levels of creatinine and uric acid.

There was a significant increase ($p < 0.05$) in urea concentration (Table 5) in the NaF-exposed Group D animals, which received a high dose of NaF. This indicates a buildup of both creatinine and urea in the body, likely due to impaired renal excretion resulting from kidney damage induced by NaF exposure, as evidenced by the severe alterations observed in the kidney histomorphological sections of animals from this group (Figure 3 and Figure 5). The increase in urea concentration in this study aligns with the findings of Emejulu et al.[34, 35] who also reported elevated urea and creatinine levels in NaF-exposed animals. However, the reduction observed with vitamin E treatment in groups E and F in the present study was not statistically significant. Electrolytes, on the other hand, play a crucial role in maintaining fluid balance between the intracellular (ICF) and extracellular (ECF) compartments, regulating blood pressure, and preserving cellular membrane potential.[36] Therefore, any imbalance in these electrolytes can become life-threatening if not corrected. The kidney is a vital organ in this regard, due to its essential functions in maintaining homeostasis and facilitating excretion, as the accumulation of excess electrolytes can be toxic to the body.

Serum electrolyte analysis (Table 6) revealed that NaF exposure can alter the concentrations of Na^+, Cl^-, and HCO_3^-, leading to disruptions in homeostasis. A significant increase ($p < 0.05$) in these electrolyte concentrations was observed in the NaF-treated groups C and D compared to the control group, indicating an electrolyte imbalance. This accumulation of electrolytes in the extracellular fluid suggests impaired renal excretion, likely due to kidney damage, as evidenced by the histomorphological changes observed in these

groups.

Conversely, a general decrease in the concentrations of Na^+, K^+, Cl^-, and HCO_3^- was noted in groups E and F, which received vitamin E. Although the decrease was not statistically significant in most cases, Group F animals showed a significant reduction in Cl^- levels, suggesting the potential of vitamin E to ameliorate electrolyte imbalance and help restore homeostasis between the intracellular and extracellular compartments following NaF exposure.

The primary extracellular cation in animals and humans, Na^+, plays key roles in nutrient and waste transport and osmoregulation. Its significant increase in NaF-exposed animals may indicate impaired renal excretion due to kidney damage or an elevated systemic Na^+ level resulting from NaF dissociation upon administration. The significant rise in Cl^- in Group C also suggests that NaF exposure affects chloride's role in cellular homeostasis and neuronal action potential transmission, with renal excretory failure likely contributing to this increase.

HCO_3^-, a major component of the body's pH buffering system responsible for maintaining acid–base homeostasis was significantly elevated in Group D, indicating a possible disruption in the buffering system and a predisposition to acidosis. Therefore, this study suggests that cell membrane potential which is primarily maintained by potassium gradients and membrane permeability, with a key contribution from the Na^+/K^+ pump may be altered following NaF exposure. This presents an avenue for further investigation.

Histomorphological examinations of kidney sections (H&E) revealed that the control groups (A and B) maintained normal renal cytoarchitecture (Figure 2). However, the kidneys of animals treated with NaF at 5 mg/kg and 20 mg/kg, respectively. Figure 2 exhibited distinct histopathological alterations compared with the control groups. These included granular dystrophy of renal tubules, necrosis of endothelial cells around the glomerulus, and invagination of epithelial cells into the lumen of the proximal and distal convoluted tubules. These findings were consistent with previous studies by Dimcevici et al.[37] and Sahu et al.[38] that reported significant structural damage to renal tissue following NaF exposure. In contrast, animals in groups E and F, which received both NaF and vitamin E (Figure 3), showed only mild distortions in renal cytoarchitecture compared to the severe changes observed in groups C and D and the control groups. This suggests that vitamin E supplementation exerted an ameliorative effect, likely through its radical scavenging activity and its support of cellular defense mechanisms.

Further analysis using Masson's trichrome stain was conducted to investigate collagen distribution. Collagen is a crucial structural component of the kidney, supporting the renal parenchyma and forming part of the basement membrane.[39,40] The control groups (A and B) exhibited normal collagen staining around the glomeruli and basement membrane. In contrast, the NaF-only groups (C and D) showed a marked reduction in collagen staining intensity (Figure 4) compared to the treated groups, indicating possible inhibition of collagen synthesis and disruption of renal structural integrity. These findings align with previous studies by Zeisberg and Neilson[41] and Park et al.[42] Conversely, the vitamin E-treated groups (E and F) demonstrated more pronounced collagen staining than both the control and NaF-only groups, suggesting enhanced collagen synthesis and the initiation of tissue repair or remodeling.

It is important to note that this study was limited to qualitative analysis using light microscopy. Nonetheless, the antioxidant properties of vitamin E are likely responsible for counteracting the NaF-induced changes, thereby preserving kidney histoarchitecture and promoting connective tissue restoration. We recommend further research employing electron microscopy, quantitative grading of tissue damage with appropriate histological scoring, and measurement of collagen stain intensity to provide a more comprehensive assessment.

5 Conclusion

Our study confirms that NaF causes histomorphological alterations in kidney cytoarchitecture and also significant changes in kidney morphometry. These changes can lead to substantial changes in the biochemical markers of renal function. Vitamin E supplementation at the tolerable UL can significantly mitigate the changes and enhance collagen stain.

Declarations

Acknowledgments

The authors wish to acknowledge the Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, for granting permission to use their facilities.

Artificial Intelligence Disclosure

The authors confirm that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

Authors' Contributions

David Jerry Akasi carried out the research and wrote the manuscript. Peter Aniekan Imo designed the experiment. Igiri Anozeng Oyono supervised the research and reviewed the manuscript.

Availability of Data and Materials

Data supporting this research, including histopathological images, can be provided on request.

Conflict of Interest
The authors declare no conflict of interest.

Consent for Publication
Not applicable.

Ethical Considerations
Care and treatment of animals were approved, and practices were performed according to approval of the Faculty of Basic Medical Science Research Committee, University of Uyo, Uyo, Nigeria, under the Code of Ethics 15/PG/BMS/AN/003. Maintenance and care of experimental animals were in line with the National Institute of Health Guidelines for humane use of laboratory animals. Protocols and recommendations were strictly adhered to.

Funding
No funding was received for this research either from public or private organizations.

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