

# **L-arginine and lisinopril supplementation protects against sodium fluoride-induced nephrotoxicity and hypertension by suppressing mineralocorticoid receptor and angiotensin-converting enzyme 3 activity**

Temitayo Olabisi Ajibade<sup>1</sup>, Olusola Adedayo Awodele<sup>2</sup>, Monsuru Oladunjoye Tijani<sup>3</sup>, Olumuyiwa Abiola Adejumobi<sup>4</sup>, Moses Olusola Adetona<sup>5</sup>, Ademola Adetokunbo Oyagbemi<sup>1,\*</sup>, Aduragbenro Deborah Adedapo<sup>6</sup>, Temidayo Olutayo Omobowale<sup>4</sup>, Abimbola Obemisola Aro<sup>7</sup>, Olufunke Eunice Ola-Davies<sup>1</sup>, Adebowale Benard Saba<sup>8</sup>, Adeolu Alex Adedapo<sup>8</sup>, Sanah Malomile Nkadimeng<sup>9</sup>, Lyndy Joy McGaw<sup>9</sup>, Prudence Ngalula Kayoka-Kabongo<sup>7</sup>, Oluwafemi Omoniyi Oguntibeju<sup>10</sup>, Momoh Audu Yakubu<sup>11</sup>

<sup>1</sup> Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

<sup>2</sup> Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Nigeria

<sup>3</sup> Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

<sup>4</sup> Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

<sup>5</sup> Department of Anatomy, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria

<sup>6</sup> Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria

<sup>7</sup> Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Florida, South Africa

<sup>8</sup> Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

<sup>9</sup> Phytomedicine Programme, Department of Paraclinical Science, Faculty of Veterinary Science, University of Pretoria, Old Soutpan Road, Pretoria, Onderstepoort 0110, South Africa

<sup>10</sup> Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa

<sup>11</sup> Department of Environmental & Interdisciplinary Sciences, College of Science, Engineering & Technology, Texas Southern University, Houston, TX, USA

\*Correspondence to: Ademola Adetokunbo Oyagbemi. Email: ademola.oyagbemi778@gmail.com

## **Abstract**

Sodium fluoride (NaF) is one of the neglected environmental toxicants that has continued to silently cause toxicity to both humans and animals. NaF is universally present in water, soil, and atmosphere. The persistent and alarming rate of increase in cardiovascular and renal diseases caused by chemicals such as NaF in mammalian tissues has led to the use of various drugs for the treatment of these diseases. The present study aimed at evaluating the renoprotective and antihypertensive effects of L-arginine against NaF-induced nephrotoxicity.

Thirty male Wistar rats (150–180 g) were used in this study. The rats were randomly divided into five groups of six rats each as follows: Control, NaF (300 ppm), NaF + L-arginine (100 mg/kg), NaF + L-arginine (200 mg/kg), and NaF + lisinopril (10 mg/kg). Histopathological examination and immunohistochemistry of renal angiotensin-converting enzyme (ACE) and mineralocorticoid receptor (MCR) were performed. Markers of renal damage, oxidative stress, antioxidant defense system, and blood pressure parameters were determined. L-arginine and lisinopril significantly ( $P < 0.05$ ) ameliorated the hypertensive effects of NaF. The systolic, diastolic, and mean arterial blood pressure of the treated groups were significantly ( $P < 0.05$ ) reduced compared with the hypertensive group. This finding was concurrent with significantly increased serum bioavailability of nitric oxide in the hypertensive rats treated with L-arginine and lisinopril. Also, there was a significant reduction in the level of blood urea nitrogen and creatinine of hypertensive rats treated with L-arginine and lisinopril. There was a significant ( $P < 0.05$ ) reduction in markers of oxidative stress such as malondialdehyde and protein carbonyl and concurrent increase in the levels of antioxidant enzymes in the kidney of hypertensive rats treated with L-arginine and lisinopril. The results of this study suggest that L-arginine and lisinopril normalized blood pressure, reduced oxidative stress, and the expression of renal ACE and mineralocorticoid receptor, and improved nitric oxide production. Thus, L-arginine holds promise as a potential therapy against hypertension and renal damage.

**Keywords:** Sodium fluoride toxicity · Oxidative stress · Nephrotoxicity · Hypertension · L-arginine, Lisinopril

## Introduction

Sodium fluoride (NaF) is one of the neglected environmental toxicants that has continued to cause toxicity to both humans and animals (Oyagbemi et al. 2021). NaF is universally present in water, soil, and the atmosphere (Oyagbemi et al. 2020). Human activity including massive global industrialization such as the industrial and pharmaceutical products and other sources has also contributed significantly to the presence of NaF in the environment (Irigoyen-Camacho et al. 2016; Choubisa and Choubisa 2016; Said et al. 2020). However, water-borne fluoride has been documented to represent the largest single component of NaF element's daily intake (Catani et al. 2007; Molina-Frechero et al. 2012). Dental fluorosis has been observed to occur normally from excess fluoride ingestion during tooth formation (Aoba and Fejerskov 2002). However, other parts of the tooth such as the enamel and dentine can be affected by fluorosis resulting from fluoride exposure that occurs during childhood (Akpata 2001; DenBesten and Li 2011).

From our laboratory, we have documented organ and non-organ toxicities associated with NaF (Oyagbemi et al. 2021, 2017; Omóbòwálé et al. 2018). Cardiovascular and neurodegenerative dysfunctions such as hypertension and motor in-coordination have also been reported (Oyagbemi et al. 2021, 2020, 2018a, 2018b, 2018c, 2017; Omóbòwálé et al. 2018). Previous findings have reported generation of reactive oxygen species (ROS) and induction of oxidative stress as mechanism of action of fluoride-induced toxicity (Oyagbemi et al. 2017; Suzuki et al. 2015; Wu et al. 2015; Shuhua et al. 2012; Izquierdo-Vega et al. 2011).

L-arginine is one of the most metabolically versatile amino acids (Gad 2010). L-arginine is known to participate in the synthesis of nitric oxide and serves as a precursor for the synthesis of polyamines, proline, glutamate, creatine, agmatine, and urea (Viribay et al. 2020). Several human and experimental animal studies have indicated that exogenous L-arginine intake has

multiple beneficial biological and pharmacological effects (Pahlavani et al. 2017; Dumont et al. 2001). Meta-analysis provides further evidence that oral L-arginine supplementation significantly lowers both systolic and diastolic blood pressure (Viribay et al. 2020; Dong et al. 2011). Nitric oxide (NO) is a well-known vasodilator produced by the vascular endothelium via the enzyme endothelial nitric oxide synthase (eNOS), the house-keeping enzyme. The inadequate production of NO has been linked to elevated blood pressure (BP) in both human and animal studies and might be due to substrate inaccessibility (Khalaf et al. 2019; Tsuboi et al. 2018). L-arginine administration has been demonstrated to improve endothelial function in various disease states (McRae 2016) and improved risk factors of cardiovascular diseases (CVD) as reported by Pahlavani et al. (2014). Interestingly, L-arginine supplementation was documented to have significant effect of lowering diastolic blood pressure and prolonging gestational age in pregnancy (Zhu et al. 2013). Another amino acid, L-citrulline has been reported to improve vascular function through increased L-arginine bioavailability and NO synthesis (Figuroa et al. 2017).

ACE inhibitors are medications used to treat and manage hypertension, which is a significant risk factor for coronary disease, heart failure, stroke, and a host of other cardiovascular conditions (Mall et al. 2021). Lisinopril is a non-sulphydryl ACE inhibitor that lowers peripheral vascular resistance with a concomitant decrease in blood pressure (Mall et al. 2021). Lisinopril has now been shown to reduce mortality and cardiovascular morbidity in patients with myocardial infarction when administered as early treatment (Wihandono et al. 2021). Lisinopril produces a smooth, gradual BP reduction in hypertensive patients without affecting heart rate or cardiovascular reflexes (Wihandono et al. 2021). Lisinopril has been reported for its antioxidant (Scisciola et al. 2022), nephroprotective, and cardioprotective properties (Ruggenti 2017; Brown et al. 2021; Wihandono et al. 2021; Østergaard et al. 2021).

The present study elucidated the molecular mechanism of anti-hypertensive action of L-arginine in a toxicant-induced hypertensive and nephrotoxic rat model.

## **Material and methods**

### **Chemicals**

Sodium fluoride, lisinopril, L-arginine, xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), oxidized glutathione (GSSG), thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium hydroxide, O-dianisidine, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and 1,2-dichloro-4-nitrobenzene were purchased from Sigma (St. Louis, MO, USA). Normal goat serum and Biotinylated 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution were purchased from Elabscience Biotechnology®, China), anti-ACE polyclonal antibody (E-AB-16159: 1:500 dilution) and anti-mineralocorticoid receptor polyclonal antibody (E-AB-70261: 1:50 Dilution). All other chemicals used for this study were of analytical grade.

### **Experimental animals and design**

Thirty male Wistar rats (150–180 g) were used in this study; the rats were randomly divided into five groups of six rats per group as control, NaF (300 ppm), NaF + L-arginine (100 mg/kg), NaF + L-arginine (200 mg/kg), and NaF + lisinopril (10 mg/kg), respectively, orally for 8 days. The administration of drugs was given daily. The concentration of NaF (Oyagbemi et al. 2021) and the dosages of L-arginine (Adejare et al., 2020) and lisinopril (Oyagbemi et al. 2021) were

chosen based on the previous literature. The rats were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow ad libitum and liberally supplied with water. Body weight and kidney weight were also measured at the end of the experiment. The blood of the rats was taken on the 8th day and rats were euthanized on the 9th day. The rats were acclimatized for 2 weeks before the commencement of the experiment.

### **Ethical approval**

The study was conducted following guidelines approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan with the approval number: UIACUREC/ 19/124.

### **Blood pressure measurement**

The systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures were determined non-invasively in conscious animals by tail plethysmography using an automated blood pressure monitor (CODA S1, Kent Scientific Corporation, Connecticut, USA). The blood pressure parameters were obtained by an indirect method of blood pressure measurement as recently reported in our laboratory (Oyagbemi et al. 2019).

### **Serum preparation**

Blood was collected from the retro-orbital venous plexus. The serum was obtained from whole blood collected into anticoagulant free sample bottles following a post-collection waiting period of 60 min. Thereafter, the serum was kept at a 4 °C temperature.

### **Determination of serum markers of renal damage**

Serum creatinine and blood urea nitrogen (BUN) were determined following the manufacturer's instructions in the purchased Randox® kits (Randox® Laboratories Ardmore, UK).

### **Preparation of renal post mitochondrial fractions (PMFs)**

The kidneys were quickly excised, rinsed, weighed, and homogenized with homogenizing buffer (0.1 M phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 10,000 g for 10 min at - 4 °C.

### **Biochemical assays**

#### ***Estimation of renal oxidative stress***

The malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of renal tissues according to the method of Varshney and Kale (1990). The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation was calculated with a molar extinction coefficient of  $1.56 \times 10^5$ /M/cm. Protein carbonyl (PCO) contents in the renal tissues were measured using the method of Reznick and Packer (1994). The absorbance of the sample was measured at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH ( $2.2 \times 10^4$  cm<sup>1</sup> M<sup>1</sup>) and expressed as nmoles/mg protein while vitamin C contents were measured as earlier described (Jacques-Silva et al. 2001).

### ***Renal antioxidant status***

The superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972), with slight modification (Oyagbemi et al. 2015). The increase in absorbance at 480 nm was monitored every 30 s for 150 s. The one unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome. Reduced glutathione (GSH) was estimated by the method of Jollow et al. (1974). Glutathione peroxidase (GPx) activity was also measured according to Beutler et al. (1963). Glutathione S-transferase (GST) was estimated by the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as substrate. The protein and non-protein thiol contents were determined as described by Ellman (1959).

### ***Estimation of serum nitric oxide concentration and total protein***

The serum nitric oxide concentrations were measured spectrophotometrically at 548 nm as previously described (Olaleye et al. 2007). Protein concentration was determined by the Biuret method of Gornal et al. (1949), using bovine serum albumin (BSA) as standard.

### ***Immunohistochemistry***

Immunohistochemistry was done as described by Oyagbemi et al. (2019). Antibodies against renal ACE and mineralocorticoid receptor (MCR) were probed in the kidney using a 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The slides were subsequently dewaxed in xylene solution for 2 min and afterward, hydration was carried out in different concentrations of ethanol (100%, 90%, and 80%) for 2 min each. Antigen retrieval was performed and followed with endogenous peroxidase blocking. Goat serum (E-1R-R217A) was added to prevent nonspecific binding and the tissues were probed with primary antibodies viz-a-viz angiotensin converting enzyme polyclonal antibody (E-AB-16159: 1:500 dilution) and anti-mineralocorticoid receptor polyclonal antibody (E-AB-70261: 1:500 dilution). Thereafter, a secondary antibody labelled E-1R-R217B was added, and the slides were incubated in humidifying chamber at room temperature for 20 min. Finally, a few drops of the substrate diaminobenzidine (DAB) were added in the dark. The reaction was terminated with deionized water and slides were immersed in hematoxylin (Sigma-Aldrich, USA) for 3 s before rinsing with PBS. The slides were placed in 80%, 90%, and 100% of ethanol, and then xylene (100%) for 2 min each. Slides were removed, allowed to dry, and a DPX mountant was applied. Sections were observed with a light microscope (Leica LAS-EZ®) using Leica software application suite version 3.4 equipped with a digital camera.

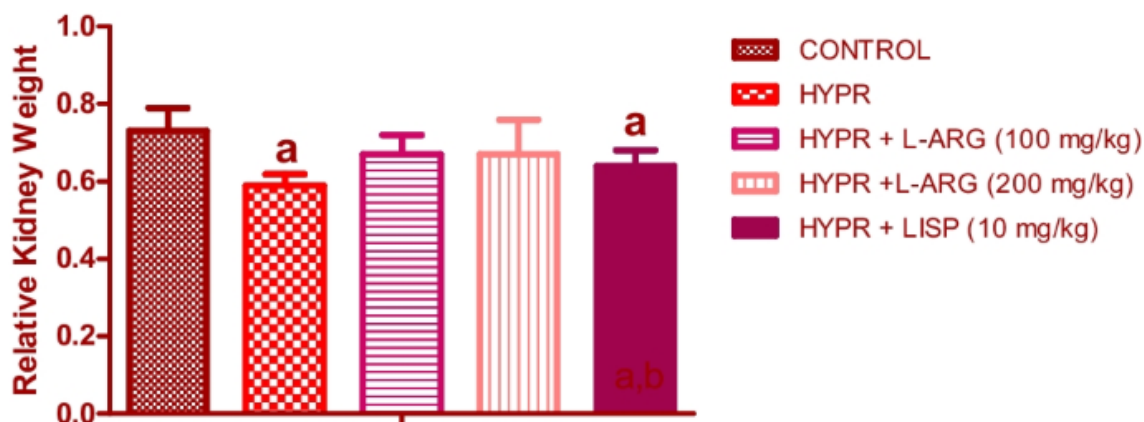
### ***Statistical analysis***

All values are expressed as mean  $\pm$  S.D. The test of significance between two groups was estimated by Student's *t* test with *P* value less than 0.05. The one-way analysis of variance (ANOVA) with Turkey's post-hoc test of Graph pad prism 5.0 was also carried out with *p*-values < 0.05 considered statistically significant (Fleiss et al. 2003).

## Results

### Sodium fluoride intoxication on body weight and kidney relative weight

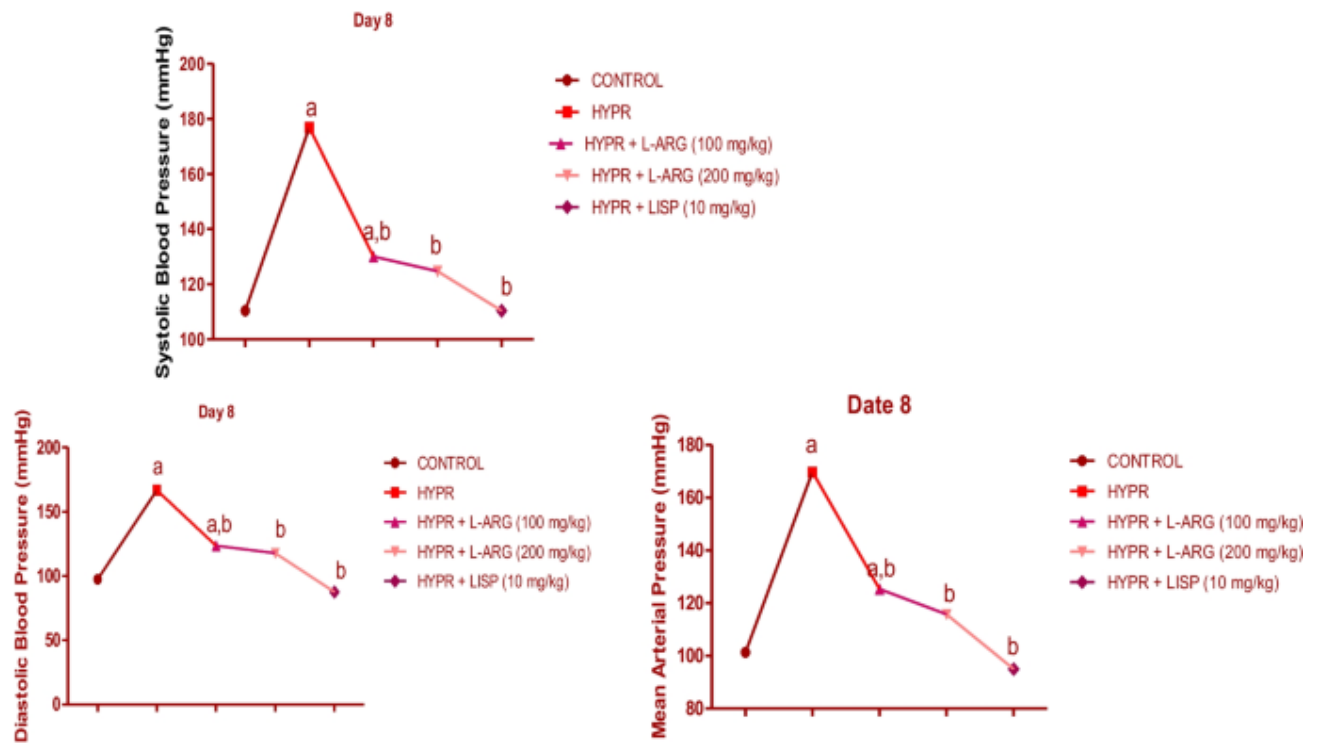
The results in Fig. 1 showed a significant ( $P < 0.05$ ) reduction in relative body weight of rats intoxicated with NaF and those co-administered with either L-arginine or lisinopril. Similarly, there was a significant ( $P < 0.05$ ) reduction in relative kidney weight of rats administered only NaF. However, L-arginine supplementation and lisinopril co-administration showed significant restorative effect on the relative kidney weight to near normal values (Fig. 1).



**Fig. 1.** Effects of L-arginine and lisinopril on body weight and relative kidney weight. Superscript (a) indicates a significant increase in serum blood urea nitrogen and creatinine compared with control at  $P < 0.05$ . Superscript (b) indicates a significant difference in systolic blood pressure compared with the hypertensive group at  $P < 0.05$ . Abbreviations: HYPR, hypertensive; L-ARG, L-arginine; LISP, lisinopril

### Hemodynamic parameters

The blood pressure parameters measured in the present study indicated significant ( $P < 0.05$ ) increases in the values of SBP, DBP, and MAP of rats intoxicated with NaF (Fig. 2). On the other hand, there was a dose-dependent reduction in the values of SBP, DBP, and MAP of rats intoxicated with NaF and treated with L-arginine, and lisinopril, respectively (Fig. 2). Lisinopril co-administration gave a better reduction of blood pressure parameters as recorded in Fig. 2.



**Fig. 2.** Systolic, diastolic and mean arterial blood pressure of hypertensive rats treated with L-arginine and lisinopril. Superscript (**a**) indicates a significant increase in when compared with control at  $P < 0.05$ . Superscript (**b**) indicates significant difference when compared with the hypertensive group at  $P < 0.05$ . Abbreviations: HYPR, hypertensive; L-ARG, L-arginine; LISP, lisinopril

**Table 1.** Effects of L-arginine supplementation on the kidney antioxidant defense system

Parameter	Control	Hypertensive	L-arginine (100 mg/kg)	L-arginine (200 mg/kg)	Lisinopril (10 mg/kg)
GPx	44.88 ± 2.84	38.84 ± 3.13 <sup>a</sup>	46.31 ± 3.74 <sup>b</sup>	45.06 ± 2.41 <sup>b</sup>	46.95 ± 5.48 <sup>b</sup>
GSH	84.77 ± 2.82	79.37 ± 4.02 <sup>a</sup>	86.68 ± 8.29	87.19 ± 4.84	88.11 ± 7.26 <sup>b</sup>
GST	91.52 ± 7.53	98.73 ± 7.93	79.90 ± 11.64 <sup>b</sup>	95.46 ± 9.29	78.54 ± 11.46 <sup>b</sup>
SOD	35.28 ± 3.32	39.63 ± 1.98 <sup>a</sup>	35.05 ± 3.47 <sup>b</sup>	33.20 ± 2.53 <sup>b</sup>	33.72 ± 2.68 <sup>b</sup>
PSH	94.00 ± 12.95	104.90 ± 12.89	179.16 ± 17.79 <sup>a,b</sup>	138.40 ± 12.70 <sup>a,b</sup>	193.79 ± 2.05 <sup>a,b</sup>
NPSH	70.52 ± 2.52	73.03 ± 5.22	69.74 ± 3.31	77.37 ± 7.34 <sup>a</sup>	63.70 ± 6.88 <sup>b</sup>
VIT c	26.93 ± 3.18	21.94 ± 2.29 <sup>a</sup>	18.57 ± 1.10 <sup>a</sup>	21.47 ± 5.72 <sup>a</sup>	23.36 ± 2.27

Values are presented as mean ± standard deviation ( $n=5$ ). <sup>a</sup>Significant difference when compared with control at  $P < 0.05$ , while superscript <sup>b</sup>Significant difference compared with hypertensive group at  $P < 0.05$

Abbreviations and units: *GPx*, glutathione peroxidase (units/mg protein); *GSH*, reduced glutathione ( $\mu\text{mol/mg}$  protein); *GST*, glutathione S-transferase (nmol 1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); *SOD*, superoxide dismutase (units/mg protein); *PSH*, protein thiol ( $\mu\text{mol/mg}$  protein); *NPSH*, non-protein thiol ( $\mu\text{mol/mg}$  protein); *VIT C*, vitamin C ( $\mu\text{mol/mg}$  protein)

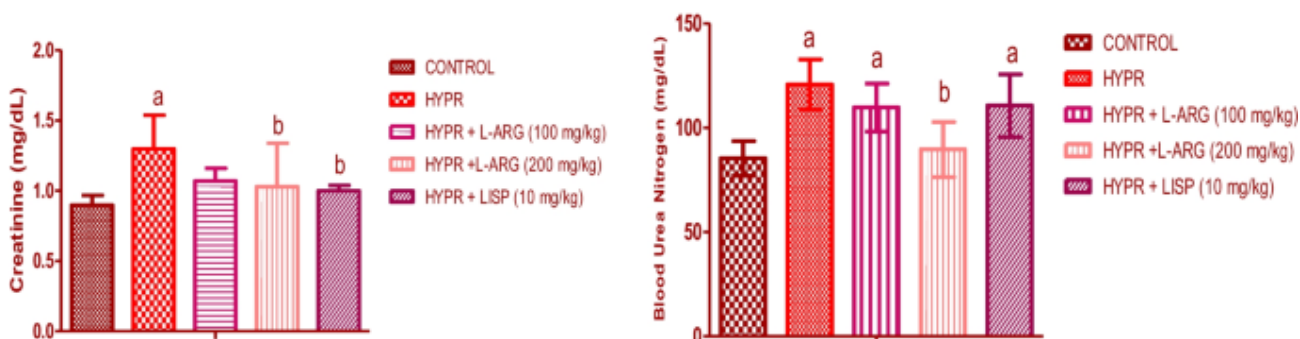
### Renal antioxidant defense system

From Table 1, 200 mg/kg of L-arginine and lisinopril supplementation was found to significantly improve the activities of renal GPx, GSH, PSH, and NPSH content, respectively. Our results showed that NaF intoxication however significantly ( $P < 0.05$ ) increased the activities of renal GST and SOD in comparison to the control (Table 1). It was interesting to observe that there was no appreciable improvement in the renal content of vitamin C except in

the rats administered lisinopril (Table 1). It is worth noting that treatment with lisinopril gave better improvement renal antioxidant defense systems (Table 1).

### Markers of renal damage and oxidative stress

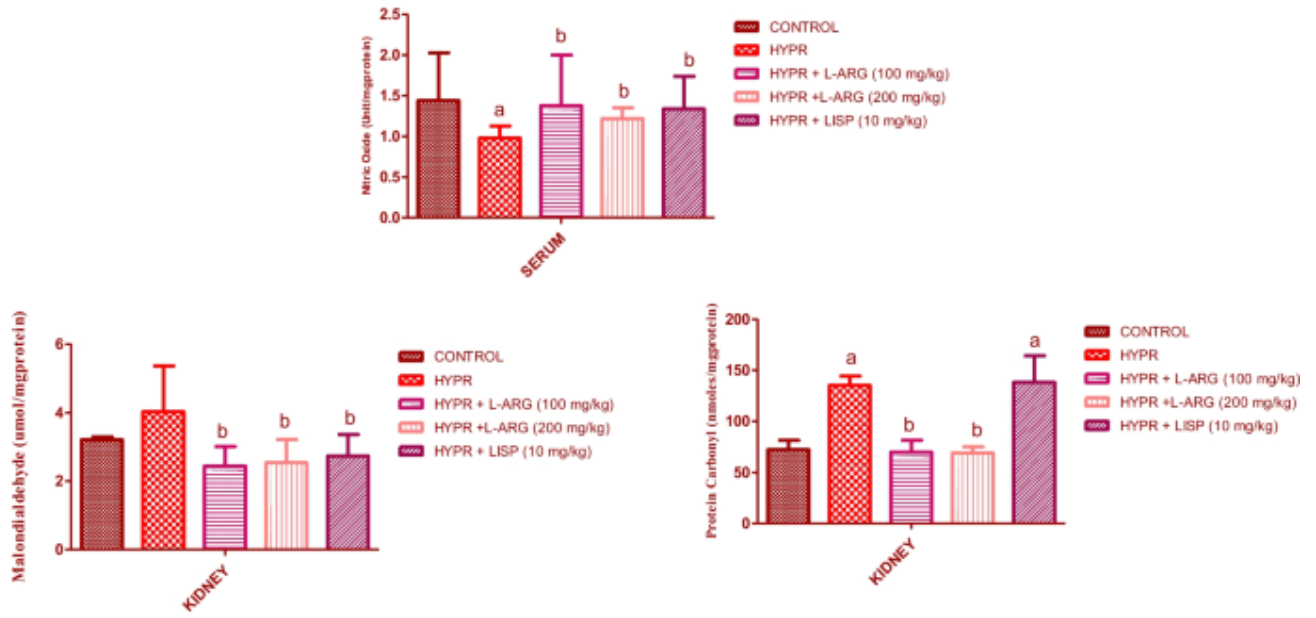
We also observed that intoxication with NaF caused a significant ( $P < 0.05$ ) increase in the values of serum BUN and creatinine when compared to the control and rats co-administered with L-arginine (100 mg/kg and 200 mg/kg) as indicated in Fig. 3. The nephron-protective effect of L-arginine and lisinopril was demonstrated as indicated by a significant ( $P < 0.05$ ) reduction in the serum levels of BUN and creatinine in comparison to the NaF-intoxicated group (Fig. 3).



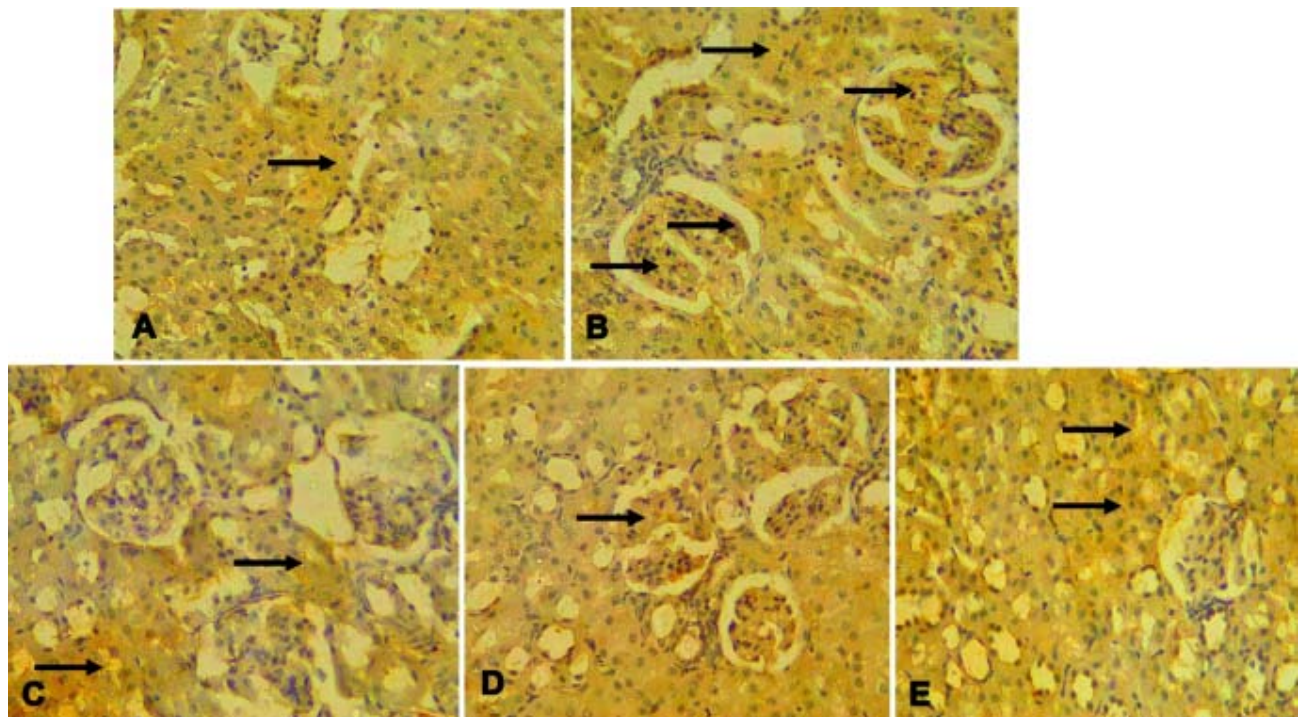
**Fig. 3.** Effects of L-arginine and lisinopril on serum blood urea nitrogen (BUN) and creatinine. Superscript (a) indicates a significant increase in when compared with control at  $P < 0.05$ . Superscript (b) indicates significant difference when compared with the hypertensive group at  $P < 0.05$ . Abbreviations: HYPR, hypertensive; L-ARG, L-arginine; LISP, lisinopril

In Fig. 4, renal MDA which is the product of lipid peroxidation, in NaF-intoxicated rats, increased significantly as compared to the control group. There was a significant ( $P < 0.05$ ) reduction in the MDA content of L-arginine and lisinopril co-administered rats when compared to the NaF alone-treated rats (Fig. 4). Our data also revealed an exaggerated increase in the content of PCO in NaF only-administered rats in comparison to the control (Fig. 4). The free radical scavenging action of L-arginine and lisinopril was demonstrated by a significant ( $P < 0.05$ ) reduction in the content of renal PCO when compared to NaF only (Fig. 4). Also in Fig. 4, the administration of NaF caused a significant ( $P < 0.05$ ) reduction in NO bioavailability relative to the control. Again, L-arginine supplementation caused a significant ( $P < 0.05$ ) improvement in NO bioavailability similar to that of lisinopril (Fig. 4).





**Fig. 4.** Effects of L-arginine and lisinopril on renal markers of oxidative stress and serum nitric oxide. Superscript (a) indicates a significant increase in when compared with control at  $P < 0.05$ . Superscript (b) indicates significant difference when compared with the hypertensive group at  $P < 0.05$ . Abbreviations: HYPR, hypertensive; L-ARG, L-arginine; LISP, lisinopril

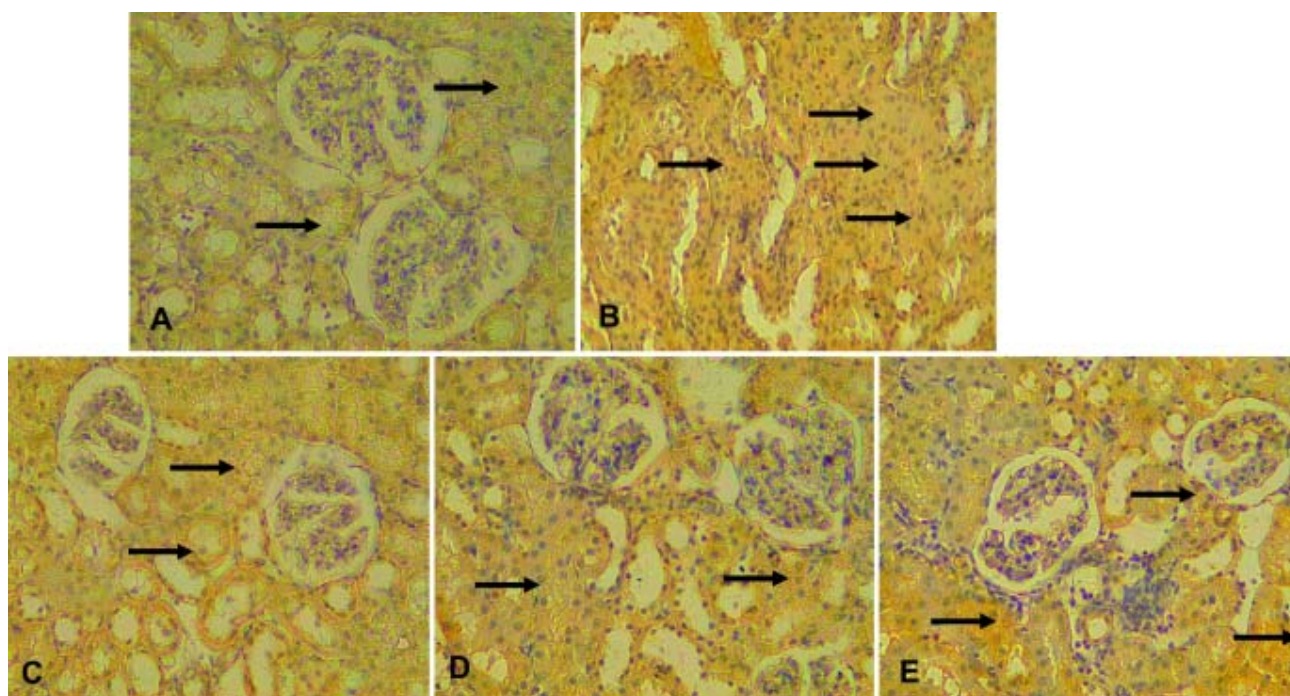


**Fig. 5.** The immunohistochemistry of renal mineralocorticoid receptor (MCR). Group A (control), Group B (HYPR (NaF; 300 ppm), Group C (HYPR + L-ARG 100 mg/kg), Group D (HYPR + L-ARG 200 mg/kg), and Group E (HYPR + LISP 10 mg/kg). Slides stained with high definition Heamtoxylin (magnification  $\times 400$ ). Abbreviations: HYPR, hypertensive; L-ARG, L-arginine; LISP, lisinopril

## Histopathology and immunohistochemistry

The histopathology of the kidney revealed mild tubular necrosis in rats intoxicated with NaF, while rats co-administered with L-arginine showed minute tubular necrosis, and no visible lesion was observed in lisinopril-treated group (Fig. 5). The renal immunohistochemistry of MCR revealed a higher expression of MCR in NaF-intoxicated rats relative to the control (Fig. 5). However, lower expression of MCR was observed in L-arginine and lisinopril-treated rats when compared to the NaF-alone rats (Fig. 5). It is important to note that lower expression of MCR was recorded in rats that received 100 mg/kg of L-arginine relative to rats that received 200 mg/kg of L-arginine and lisinopril (Fig. 5).

In another experiment, our study revealed higher expression of ACE in renal tissues of rats intoxicated with NaF when compared to the control (Fig. 6). Interestingly, co-treatment with either L-arginine or lisinopril reduced the expression of ACE relative to the NaF-intoxicated rats (Fig. 6).



**Fig. 6.** Group **A** (Control), Group **B** (HYPR (NaF; 300 ppm), Group **C** (HYPR + L-ARG 100 mg/kg), Group **D** (HYPR + L-ARG 200 mg/kg), and Group **E** (HYPR + LISP 10 mg/kg). Slides stained with high definition Hematoxylin (magnification  $\times 400$ ). Abbreviations: HYPR, hypertensive; L-ARG, L-arginine; LISP, Lisinopril

## Discussion

This study showed that L-arginine and lisinopril ameliorated NaF-induced hypertension in male Wistar rats. This can be corroborated by a statistically significant reduction in high SBP, DBP, and MAP across the treated groups when compared with the hypertensive untreated rats. Our findings also confirmed earlier reports on the toxicity of NaF on cardiovascular system (Oyagbemi et al. 2021, 2018a, 2018b, 2018c, 2017; Omóbòwálé et al. 2018). Administration of NaF alone to rats led to a significant decrease in serum NO bioavailability in the hypertensive

group. However, rats in the treated groups (L-arginine or lisinopril) had a noticeable increase in NO availability. The reduction in NO bioavailability has been reported to be involved in the pathogenesis of hypertensive conditions (Elmarakby and Sullivan 2021; Stamm et al. 2021; Travis et al. 2021) and other cardiac complications through generation of ROS (Oyagbemi et al. 2021, 2017). L-arginine is a precursor for the synthesis of NO (Almannai and El-Hattab 2021; Ma 2021; Yaremchuk et al. 2021), and the NO produced from vascular endothelium helps to maintain a continuous tone that is essential for the regulation of blood flow, blood pressure, platelet aggregation, and vasodilation (Umnyagina et al. 2021; Pautz et al. 2021). It was evident from our study that L-arginine or lisinopril significantly increased NO bioavailability and reversed high blood pressure precipitated by NaF intoxication.

We observed from our study that NaF intoxication caused significant increase in blood urea nitrogen (BUN) and creatinine levels. The increase in BUN and creatinine has been associated with various degrees of renal injuries (Chen et al. 2021a, b; Ni et al. 2021; Nasiruddin et al. 2020). The observed nephrotoxicity by NaF might be due to free radical generation and increased protein catabolism with concomitant systemic oxidative damage. This finding might also suggest extensive glomerular and tubular epithelial cell damage observed in the histopathology are positively correlated with exaggerated levels of BUN and creatinine. Treatment with L-arginine or lisinopril significantly attenuated these deleterious effects by the reduction in BUN and creatinine levels across treated groups in comparison to the NaF-intoxicated group. This therefore indicates the nephroprotective effect of L-arginine or lisinopril against nephrotoxicity induced by NaF intoxication. Our study therefore is in support of nephroprotective effect of L-arginine against nephrotoxicity and hepatorenal damage (Saka et al. 2021; Abdelhalim et al. 2018). The use of function foods and Cr-methionine has documented against oxidative stress in animals (Hoan et al., 2021; Bin-Jumah et al. 2020; Abdelnour et al. 2019).

The ability of L-arginine to mitigate oxidative stress in hypertensive rats was also demonstrated in the present study. Renal markers of oxidative stress including hydrogen peroxide ( $H_2O_2$ ) generated, MDA, and PCO contents increased significantly in NaF-induced hypertensive rats compared with the control. The exaggerated production of  $H_2O_2$  as classic example of ROS that has been reported during oxidative stress with ultimate damage to proteins, nucleic acids, cell membranes has been implicated in the development of some diseases (Yang et al. 2021; Yu et al. 2021; Zhang et al. 2021). The generated  $H_2O_2$  can react with superoxide anion radical ( $O_2^{\cdot-}$ ) to initiate the Haber-Weis reaction, thereby producing hydroxyl radical ( $\cdot OH$ ). It was exciting to observe a significant reduction in  $H_2O_2$  content in rats co-administered with L-arginine or lisinopril. The ability of L-arginine or lisinopril to reduce the renal content of  $H_2O_2$  was an indication of free radical scavenging action of L-arginine.

Malondialdehyde (MDA) is one of the final products of peroxidation of polyunsaturated fatty acids (PUFA) in the cell (Wang et al. 2021; Torun et al. 2009; Gawel et al. 2004). The MDA is a toxic aldehyde that can initiate oxidative cellular damage in both target and non-target tissues (Morelli et al. 2021). In this study, NaF intoxication significantly increased the content of renal MDA. However, anti-oxidative action of L-arginine or lisinopril was demonstrated as shown in the reduction in aforementioned exaggerated production of renal MDA. Protein oxidation, and their level in tissues and plasma, has been reported as a relatively stable marker of oxidative damage (Dayanand et al. 2012). In fact, pathogenesis and pathophysiology of many disease conditions have been associated with increased protein carbonyl content (Akinrinde et al. 2021; Marques et al. 2021; Ommati et al. 2021; Rodríguez-Sánchez et al. 2021). From this study, L-arginine's protection against NaF-induced renal protein

carbonylation might be associated with the antioxidant activity of L-arginine or lisinopril which prevents protein oxidation. Protein carbonylation, one of the most harmful irreversible oxidative protein modifications, has been considered a major hallmark of oxidative stress-related disorders including aging and several age-related disorders (Fedorova et al. 2014). From this study, we can propose that L-arginine could be found applicable in the management of aging and several age-related disorders against protein oxidation and crosslinking.

Glutathione in its reduced form is an important intracellular antioxidant that protects against a variety of oxidant species (Masella et al. 2005). The protective mechanisms of glutathione against oxidative stress which can be through detoxification of enzymes such as glutathione peroxidase against oxidative stress, scavenging hydroxyl radicals, and singlet oxygen directly (Masella et al. 2005). Glutathione peroxidase (GPx) is a selenium-containing enzyme that catalyzes detoxification of lipid hydroperoxide and hydrogen peroxides to water and oxygen (O<sub>2</sub>). The reduction in the activity of GPx could lead to a concurrent increase in hydrogen peroxide with subsequent tissue damage (Farhat et al. 2018; Espinoza et al. 2008). Superoxide dismutase (SOD), on the other hand, catalyzes the dismutation of the superoxide anion radical to hydrogen peroxide (Pizzino et al. 2017).

Our data also showed a significant decrease in the activity of enzymatic and non-enzymatic antioxidants such as GPx, SOD, reduced GSH, and vitamin C in NaF-intoxicated hypertensive group, confirming the involvement of oxidative stress in the pathogenesis of hypertension. Treatment of the hypertensive rats with L-arginine at 100 mg/kg and 200 mg/kg brought about significant improvement in the antioxidant defense system. However, the increase in GSH level in the renal tissues of the hypertensive rats treated with L-arginine was not significant except in the group treated with 10 mg/kg lisinopril. The reduction in the levels of markers of oxidative stress and concurrent increase in antioxidant enzymes might suggest an ability of L-arginine to scavenge free radicals and mitigate oxidative stress associated with NaF toxicity.

The significant decrease in the activity of SOD and GPx in the hypertensive group may subsequently lead to an increase in superoxide anion radical and H<sub>2</sub>O<sub>2</sub> levels, thereby potentiating oxidative stress as a major factor in the progression of hypertension. The accumulation of the superoxide anion radical was also a sequel to the observed decrease in the activity of SOD. Hence, increasing levels of the superoxide anion radical might enhance the uncoupling of eNOS with a resultant reduction in NO bioavailability. Furthermore, superoxide anion radical is also capable of reacting with NO to form peroxynitrite, which is a cytotoxic signaling molecule (Wu et al. 2020; Hu et al. 2019). Thus, the observable increase in the activity of GPx in the kidney tissues is suggestive of antioxidant and ameliorative roles of L-arginine against NaF toxicity.

The over-activation of MCR in animal models of chronic kidney disease (CKD) has been reported to play significant roles in the pathophysiology and pathogenesis of cardiorenal dysfunctions including inflammation and fibrosis in the kidneys and hearts and increased sodium retention and hypertension (Georgianos and Agarwal 2021). MCR antagonists have become novel therapeutic interventions to retard the progression of CKD with attendant improvement in cardiovascular morbidity and mortality (Droebner et al. 2021; Kovarik et al. 2021; Patrono and Volpe 2021). Our study revealed an over-activation of MCR by NaF intoxication as recorded with higher expression of renal MCR. The observed higher expression of MCR could be positively correlated with exaggerated high blood pressure obtained in rats administered only NaF. From our data, L-arginine or lisinopril co-administration with NaF caused a reduction in the expression of MCR. This might be indicative of renoprotective and

anti-hypertensive action of L-arginine and lisinopril, respectively. The amino acid L-arginine could be found applicable for the management of toxicant-induced nephrotoxicity.

Recently, science has taken the advantage of selectively inhibiting ACE as a therapeutic target for preventing CKD and better management of hypertension (Puspita et al. 2021; Bas 2021; Alves-Lopes et al. 2021; Chen et al. 2021a, b). In this study, we also investigated renal immunolocalization of ACE following NaF intoxication. The immunohistochemistry revealed a higher expression of renal ACE in rats administered with NaF relative to the control and rats co-administered with either L-arginine or lisinopril. The increased expression of ACE was similar to that of MCR as stated above, meaning that NaF nephrotoxicity might be through over-activation of MCR and ACE signaling pathways. The over-activation of these pathways could actually be responsible for the nephrotoxicity and hypertension. The ability of L-arginine to block the activities of MCR and ACE could be maximized as a novel therapeutic agent in the management and treatment of kidney damage and associated hypertension.

## **Conclusion**

The results of this study showed that 200 mg/kg of L-arginine normalized high blood pressure, reduced oxidative stress, improved renal antioxidant defense system, offered protection against renal damage and nephrotoxicity, and improved nitric oxide bioavailability thereby serving as a precursor to nitric oxide production. Thus, L-arginine could serve as a potential alternative therapy against toxicant-induced oxidative stress, nephrotoxicity, and hypertension via increase in the supply of endogenous nitric oxide.

## **Data availability**

Data will be made available on request.

## **Acknowledgements**

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## **Contributions**

The authors, Ademola Adetokunbo Oyagabemi, Olusola Adedayo Awodele, and Temidayo Olutayo Omobowale, designed the experiment. Histopathology was carried out by Monsuru Oladunjoye Tijani. The blood pressure was performed by Temitayo Olabisi Ajibade, Olumuyiwa Abiola Adejumobi, and Temidayo Olutayo Omobowale. Olusola Adedayo Awodele and Ademola Adetokunbo Oyagabemi performed the immunohistochemistry and biochemical assays Moses Olusola Adetona, Aduragbenro Deborah A. Adedapo, Temidayo Olutayo Omobowale, Abimbola Obemisola Aro, Olufunke Eunice Ola-Davies, Adebowale Benard Saba, Adeolu Alex Adedapo, Sanah Malomile Nkadimeng, Lyndy Joy McGaw, Prudence Ngalula Kayoka-Kabongo, Oluwafemi Omoniyi Oguntibeju, and Momoh Audu Yakubu supervised, proofread, and approved the submission.

The study was conducted following guidelines approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan (Approval number: UIACUREC/19/124).

## Consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

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