L- Arginine Supplementation Protections Sodium Fluoride-Induced Nephrotoxicity And Hypertension By Suppressing Mineralocorticoid Receptor and Angiotensin Converting Enzyme Activity

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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 the atmosphere. The persistent and alarming rate of increase in cardiovascular and renal diseases and disorders caused by chemicals such as sodium fluoride (NaF) in mammalian tissues have led to the use of various drugs for the treatment of these diseases. This study aims at evaluating the renoprotective and antihypertensive effects of L- Arginine on 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 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 eight days. Histopathological examination and immunohistochemistry of renal angiotensin converting enzyme (ACE) and mineralocorticoid receptor (MCR) were performed. Markers of renal damage, oxidative stress, antioxidant defence system, and blood pressure parameters were determined. L- Arginine 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 treated groups. Also, there was significant reduction in the level of blood urea nitrogen (BUN) and creatinine in the serum of the hypertensive rats treated with L- arginine. There was significant ($p<0.05$) reduction in markers of oxidative stress such as hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and protein carbonyl (PCO) and concurrent increase in the levels of antioxidant enzymes in the kidney of hypertensive rats treated with L arginine. The results of this study suggest that L- Arginine normalized high blood pressure, reduced oxidative stress, reduced the expression of renal ACE and MCR, and improved nitric oxide production. Thus, L- Arginine holds promise as a potential therapy against hypertension and renal damage.

Introduction

Sodium fluoride (NaF) is one of the neglected environmental toxicants that has continued to silently 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 have 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; DenBesten and Wu 2011). However, other parts of the tooth such as the enamel and dentine can be affected by fluorosis resulting from fluoride exposure occuring during childhood (Akpata 2001; DenBesten and Wu 2011).

From our laboratory, we have documented organ and non-organ toxicities associated with NaF (Oyagbemi et al. 2017; Omóbòwálé et al. 2018; Oyagbemi et al. Oyagbemi et al. 2021). Cardiovascular

and neurodegenerative dysfunctions such as hypertension and motor in-coordination have been reported (Oyagbemi et al. 2017; Omóbòwálé et al. 2018; Oyagbemi et al. 2018a; Oyagbemi et al. 2018b; Oyagbemi et al. 2018c; Oyagbemi et al. 2021; Oyagbemi et al. 2020). Previous findings have reported generation of reactive oxygen species (ROS) and oxidative stress mechanism of action of fluoride-induced toxicity (Izquierdo-Vega et al. 2011; Shuhua et al. 2012; Suzuki et al. 2015; Wu et al. 2015; Oyagbemi et al. 2017).

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 (McConell et al. 2019; Viribay et al. 2020). Several human and experimental animal studies have indicated that exogenous L- Arginine intake has multiple beneficial biological and pharmacological effects (Dumont et al., 2001; Pahlavani et al. 2017). Meta-analysis provides further evidence that oral L- Arginine supplementation significantly lowers both systolic and diastolic blood pressure (Dong et al. 2011; Viribay et al. 2020). 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 (Tsuboi et al. 2018; Khalaf et al. 2019). L- Arginine administration has been demonstrated to improve endothelial function in various disease states (McRae 2016; McConell et al. 2019), 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 nitric oxide synthesis (Figueroa et al. 2017).

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

Material And Methods

Chemicals

Sodium fluoride, L- Arginine , xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), oxidized glutathione (GSSG), Sodium fluoride, thiobarbituric acid (TBA), trichloro acetic acid (TCA), sodium hydroxide, O-dianisidine, and hydrogen peroxide (H_2O_2), 1,2-dichloro-4-nitrobenzene, were purchased from Sigma (St. Louis, MO, USA). Normal goat serum, Biotinylated 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution were purchased from Elabscience Biotechnology®, China), anti- Angiotensin Converting Enzyme1 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 each 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 eight days. The concentration of NaF (Oyagabemi et al. 2021) and the dosages of L- Arginine (Saad 2021) and Lisinopil (Oyagabemi et al., 2021) were chosen based on the previous literature. The animals were also fed with rat cubes *ad libitum* and water was supplied liberally. 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. The blood of the rats was taken on the 8^{th} day and rats were sacrificed on the 9^{th} day.

Ethical approval

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).

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 from our laboratory (Oyagbemi et al. 2019).

Serum preparation

The serum was obtained from whole blood collected into anticoagulant free sample bottles following a post-collection waiting period of 60 mins. Thereafter, the serum was kept at a $4^{o}C$ 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, United Kingdom).

Preparation of renal post mitochondrial fractions

The kidney and testes were quickly excised, rinsed, weighed and homogenized with homogenizing buffer (0.1M phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 10,000 *g* for 10 minutes at $-4^{O}C$.

Biochemical assays

Estimation of renal oxidative stress

Hydrogen peroxide generation was determined according to the method of Wolff (1994). The reaction mixture was subsequently incubated at room temperature for 30 minutes. The mixtures were read at

absorbance of 560 nm and H_2O_2 generated was extrapolated from H_2O_2 standard curve. The Malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of cardiac and 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 1.56×10^5/M/cm. Protein carbonyl (PCO) contents in the renal and cardiac 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 (2.2 104 cm1 M1) 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.

Histopathology

Small pieces of kidney were fixed in 10% formalin, embedded in paraffin wax, and sections of 5-6 mm in thickness were made and thereafter stained with hematoxylin and eosin (H&E) for histopathological examination according to the methods as previously described (Drury et al. 1976). Thereafter, the sections were examined with light microscopy.

Immunohistochemistry

Immunohistochemistry was done as described by Oyagbemi et al. (2019). Antibodies against renal angiotensin converting enzyme (ACE) and mineralocorticoid receptor (MCR) were probed in the heart using 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The kidney samples were fixed with 10% paraformaldehyde, embedded in paraffin and sectioned at a thickness of 5 μm. The slides were subsequently dewaxed in xylene (100%) solution for 2 minutes and afterward, hydration was carried out

in different concentrations of ethanol (100%, 90%, and 80%) for 2 minutes each. The hydrated slides were rinsed and put in a PBS buffer tank for 5 mins. The antigen retrieval was performed with citrate buffer solution containing 2.1 g of citric acid monohydrate and 14.75 g of trisodium citrate dehydrate adjusted to pH 6.0 in microwave oven. Endogenous peroxide (H_2O_2 block) was carried out following manufacturer's instructions as directed on the kit (E-IR-217C). Drops of H_2O_2 were added to cover the sections and incubated in humidifying chamber at room temperature for 10 min. The slides were rinsed afterwards and put back in the PBS tank for 5 min. Goat serum (E-1R-R217A) was added onto the slides to prevent nonspecific binding and incubated in humidifying chamber at room temperature for 30 mins. After 30 mins of incubation, the tissues were probed with primary antibodies viz-a-viz Angiotensin Converting Enzyme1 polyclonal antibody (E-AB-16159: 1:500 Dilution) and anti-mineralocorticoid receptor polyclonal antibody (E-AB-70261: 1:500 Dilution), and incubated for 2 hours at room temperature. Following incubation, the slides were rinsed with PBS and secondary antibody labelled (E-1R-R217B) was added, and the slides were incubated in humidifying chamber at room temperature for 20 min. Thereafter, the slides were rinsed and immersed in PBS tank for 5 min. Finally, a few drops of the substrate diaminobenzidine (DAB) was added at room temperature for 10 s; 50 µL of DAB concentrate (E-1R-R217D) + 1 mL DAB solution (E-1R-R217E) 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 minutes 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

Data obtained were analyzed with One-way ANOVA with Dunnett's post-test at a 95% confidence limit. All values are expressed as mean ±S.D. The test of significance between two groups was estimated by Student's t test.

Results

Sodium fluoride intoxication on kidney weight and kidney relative weight

The results from figure 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 (Figure 1).

Hemodynamic parameters

The blood pressure parameters measured in the present study indicated significant ($P<0.05$) increases in the values of systolic blood pressure (SBP), diastolic blood pressure (SBP) and mean arterial pressure

(MAP) of rats intoxicated with NaF (Figure 2). On the other hand, there was dose-dependent reduction in the values of SBP, DBP and MAP of rats intoxicated with NaF and treated with L- Arginine and Lisinopril respectively (Figure 2). Lisinopril co-administration gave a better reduction of blood pressure parameters as recorded in figure 2.

Renal antioxidant defence system

From table 1, L- Arginine supplementation was found to significantly improve the activities of renal GPx, GST, SOD, PSH and vitamin C content, respectively. Our results showed that NaF intoxication significantly ($P<0.05$) reduced activities of renal GPx, GST, SOD, and vitamin C content in comparison to the control (Table 1). Furthermore, there were significant increases in the activity of renal SOD, PSH and NPSH content, but not statistically significant when compared to the control (Table 1).

In table 2, we recorded significant reductions in the activities of GPx, GST, SOD, and GSH, NPSH and vitamin C content in cardiac tissues of NaF intoxicated rats. However, L-ARG co-administration at 100 mg/kg and 200 mg/kg enhanced the activities of cardiac SOD and GPx together with the content of GSH, PSH and NPSH, respectively. It was interesting to observe that there was no appreciable improvement in the cardiac content of vitamin C except in the rats administered Lisinopril (Table 2). It is worth noting that treatment with Lisinopril gave better improvement in both cardiac and renal antioxidant defence systems (Tables 1 and 2).

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 blood urea nitrogen (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 figure 3. The nephron-protective effect of L-Arginine was demonstrated as indicated with significant reduction in the serum levels of BUN and creatinine in comparison to the NaF intoxicated group (Figure 3).

In figure 4, renal malondialdehyde (MDA) which is the product of lipid peroxidation, in NaF intoxicated rats, increased significantly as compared to the control group. There was significant reduction in the MDA content of L- Arginine and Lisinopril co-administered rats when compared to the NaF alone treated rats (Figure 4). Our data also revealed an exaggerated content of protein carbonyl (PCO) in NaF only administered rats in comparison to the control (Figure 4). The free radical scavenging action of L-Arginine was demonstrated by significant reduction in the content of renal PCO when compared to NaF only rats (Figure 4). Also in figure 4, the administration of NaF caused significant reduction in nitric oxide (NO) bioavailability relative to the control. Again, L- Arginine supplementation caused significant improvement in NO bioavailability similar to that of Lisinopril (Figure 4).

Histopathology and immunohistochemistry

The histopathology of the kidney revealed mild tubular necrosis in rats intoxicated with NaF, while rats co-administered with L- Arginine and Lisinopril showed no visible lesion (Figure 5). The renal

immunohistochemistry of mineralocorticoid receptor (MCR) revealed higher expression of MCR in NaF intoxicated rats relative to the control (Figure 6). However, lower expression of MCR was observed in L-Arginine and Lisinopril treated rats when compared to the NaF alone rats (Figure 6). It is important to note that lower expression of MCR was recorded in rats that received 100 mg/kg of L- Arginine relative to those that received 200 mg/kg of L- Arginine and Lisinopril (Figure 6).

In another experiment, our study revealed higher expression of angiotensin converting enzyme (ACE) in renal tissues of rats intoxicated with NaF when compared to the control (Figure 7). Interestingly, co-treatment with either L- Arginine or Lisinopril reduced the expression of ACE relative to the NaF intoxicated rats (Figure 7).

Discussion

This study showed that L – arginine ameliorates NaF-induced hypertension in male Wistar rats. This can be corroborated by statistically significant reduction in systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure across the treated groups when compared with the hypertensive untreated rats. Our findings also confirmed earlier reports on toxicity of NaF on cardiovascular system (Oyagbemi et al. 2017; Omóbòwálé et al. 2018; Oyagbemi et al. 2018a; Oyagbemi et al. 2018b; Oyagbemi et al. 2018c; Oyagbemi et al. 2021). Administration of NaF alone to rats led to a significant decrease in serum nitric oxide (NO) bioavailability in the hypertensive group. However, rats in the treated groups had noticeable increase in NO availability. The reduction in NO bioavailability has been reported to be involved in the pathogenesis of hypertensive conditions (Pinheiro et al. 2017; Elmarakby and Sullivan 2021; Stamm et al. 2021; Travis et al. 2021) and other cardiac complications through generation of reactive oxygen species (Oyagbemi et al. 2017; Oyagbemi et al. 2021). L- Arginine is a precursor for the synthesis of nitric oxide (Almannai et al. 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; Dosunmu-Ogunbi et al. 2021; Pautz et al. 2021). It was evident from our study that L- Arginine 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 (Nasiruddin et al. 2020; Chen et al. 2021; Ni et al. 2021). The observed nephrotoxicity by NaF might be due to free radical generation and increase protein catabolism with concomitant systemic oxidative damage. This finding might also suggest extensive glomerular damage and tubular epithelial cell damage that are positively correlated with exaggerated levels of BUN and creatinine. Surprisingly, treatment with L- Arginine significantly attenuated these deleterious effects by the reduction in BUN and creatinine levels across treated groups in comparison to the Na intoxicated group. This therefore indicates the nephropretective effect of L- Arginine against nephrotoxicity induced by NaF intoxication. Our study therefore is in support of nephropretective effect of L- Arginine against nephrotoxicity and hepatorenal damage (Abdelhalim et al. 2018; Saka et al. 2021).

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, malondialdehyde and protein carbonyl contents increased significantly in NaF-induced hypertensive rats compared with the control. The exaggerated production of H_2O_2 as classic example of reactive oxygen species (ROS) has been reported during oxidative stress with ultimate damage to proteins, nucleic acids, cell membranes and have been implicated in the development of some diseases (He et al. 2021; Yang et al. 2021; Yu et al. 2021; Zhang et al. 2021). The generated H_2O_2 can react with superoxide anion radical ($O_2^{\bullet}$) to initiate the Haber- Weis reaction, thereby producing hydroxyl radical (·OH). It was exciting to observe a significant reduction in H_2O_2 content in rats co-administered with L- arginine. The ability of L-Arginine 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 in the cell (Gawel et al. 2004; Torun et al. 2009; Wang et al. 2021). 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 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. 2020; 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 which prevents protein oxidation. Protein carbonylation, one of the most harmful irreversible oxidative protein modifications has been considered as 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 can be through detoxification of enzymes such as glutathione peroxidase against oxidative stress and 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_2). The reduction in the activity of GPx would lead to a concurrent increase in hydrogen peroxide with subsequent tissue damage (Espinoza et al. 2000; Farhat et al. 2018). Superoxide dismutase (SOD) on the other hand catalyzes the dismutation of the supeoxide anion radical to hydrogen peroxide (Pizzino et al. 2017).

Our data also showed significant decrease in the activity of enzymatic and non-enzymatic antioxidants such as glutathione peroxidase (GPx), superoxide dismutase (SOD), reduced glutathione (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 of antioxidant defence system. However, the increase in reduced glutathione 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 may 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_2O_2 levels, thereby potentiating oxidative stress as a major factor in the progression of hypertension. The accumulation of the superoxide anion radical was also sequel to the observed decrease in the activity of SOD. Hence, increasing levels of the superoxide anion radical might enhance the uncoupling of endothelial nitric oxide synthase (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 the mineralocorticoid receptor (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, increased sodium retention and hypertension (Georgianos and Agarwal 2021). MCR antagonists have become novel therapeutic intervention 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 overactivation 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 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 angiotensin converting enzyme (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. 2021). In this study, we also investigated renal immunolocalization of ACE following NaF intoxication. The immunohistochemistry revealed 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 in the 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 pathway. 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 novel therapeutic agent in the management and treatment of kidney damage and associated hypertension.

Conclusion

The results of this study showed that L- Arginine normalized high blood pressure, reduced oxidative stress, improved renal antioxidant defence 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.

Declarations

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Ethical approval

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 to Publish: Not applicable

Authors 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, Momoh Audu Yakubu supervised, proof-read and approved the submission.

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Tables

Table 1 Effects of L-Arginine supplementation on the kidney antioxidant defence 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[a]	46.31±3.74[b]	45.06±2.41[b]	46.95±5.48[b]
GSH	84.77±2.82	79.37±4.02[a]	86.68±8.29	87.19±4.84	88.11±7.26[b]
GST	91.52±7.53	98.73±7.93	79.90±11.64[b]	95.46±9.29	78.54±11.46[b]
SOD	35.28±3.32	39.63±1.98[a]	35.05±3.47[b]	33.20±2.53[b]	33.72±2.68[b]
PSH	94.00±12.95	104.90±12.89	179.16±17.79[a,b]	138.40±12.70[a,b]	193.79±2.05[a,b]
NPSH	70.52±2.52	73.03±5.22	69.74±3.31	77.37±7.34[a]	63.70±6.88[b]
VIT C	26.93±3.18	21.94±2.29[a]	18.57±1.10[a]	21.47±5.72[a]	23.36±2.27

Values are presented as mean ± standard deviation (n=5). Superscript [a] indicates significant difference when compared with control at P<0.05, while superscript [b] indicates significant difference compared with hypertensive group at P<0.05.

Abbreviations and units: GPx; Glutathione Peroxidase (units/mg protein): GSH; Reduced Glutathione (µmol/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 (µmol/mg protein): NPSH; non-protein thiol (µmol/mg protein): VIC T; Vitamin C (µmol/mg protein).

Table 2 is not available with this version.

Figures

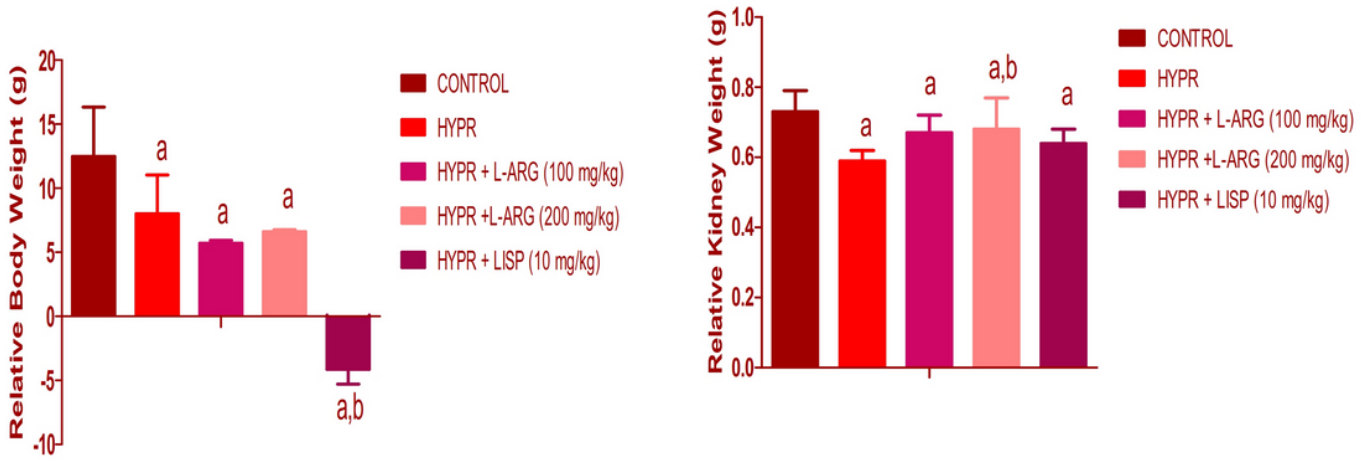

Fig. 1 Effects of L-Arginine and Lisinopril on Relative Body Weight, Relative Brain weight and Relative Kidney Weight. Superscript (a) indicates a significant increase in Serum Blood Urea Nitrogen and Creatinine compared with control at P<0.05. Superscript (b) indicates a significant decrease in systolic blood pressure compared with the hypertensive group at P<0.05. **Abbreviations:** (HYPR) Hypertensive; (L-ARG) L-Arginine; (LISP) Lisinopril.

Figure 1

See image above for figure legend.

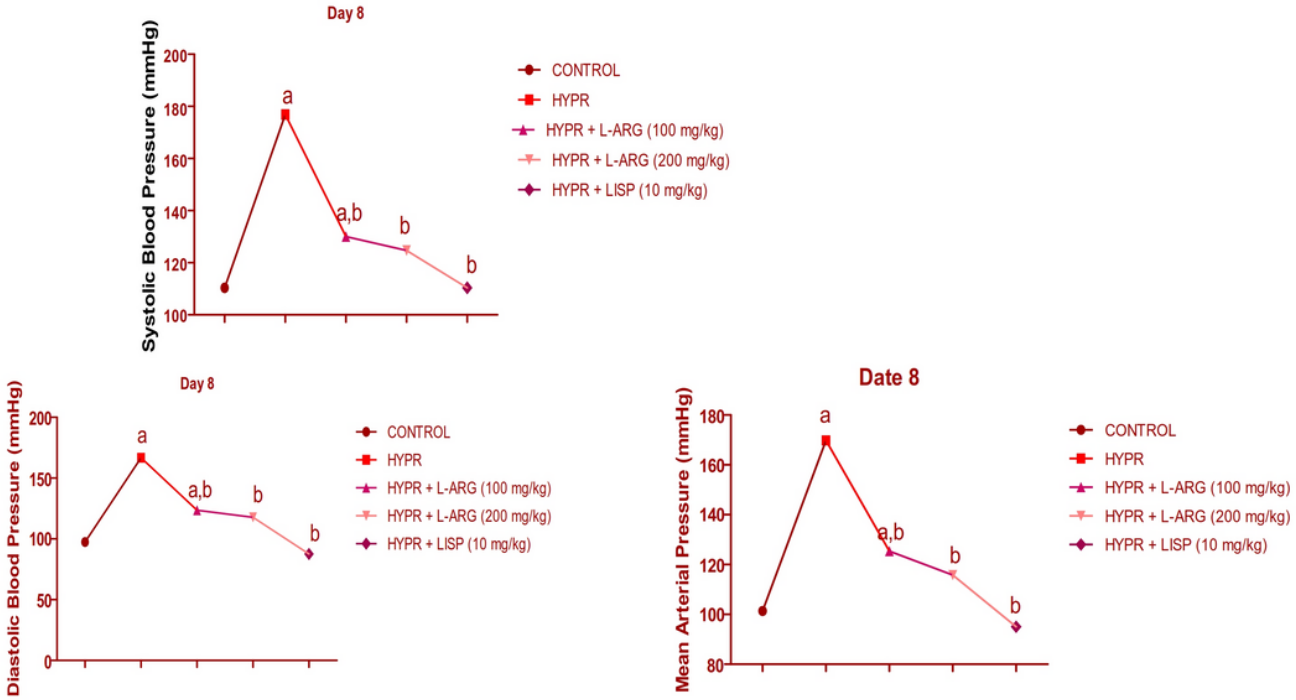

Fig. 2 Systolic, Diastolic and Mean Arterial Blood Pressure of hypertensive rats with treatment with L-Arginine and Lisinopril. Superscript (a) indicates a significant increase in when compared with control at P<0.05. Superscript (b) indicates significant increase when compared with the hypertensive group at P<0.05. **Abbreviations**: (HYPR) Hypertensive; (L-ARG) L-Arginine; (LISP) Lisinopril.

Figure 2

See image above for figure legend.

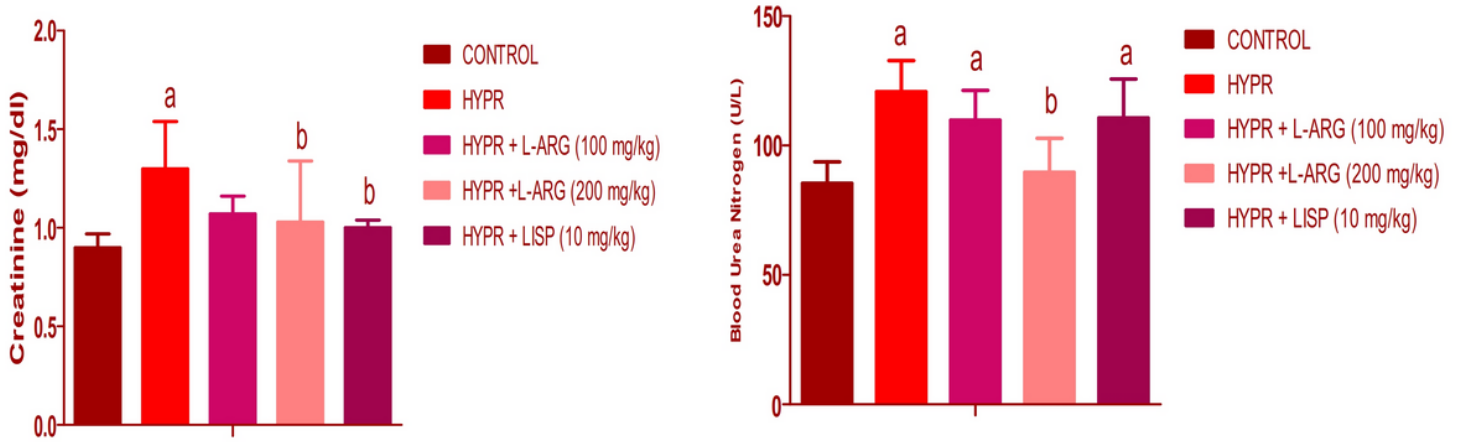

Fig. 3 Effects of L-Arginine and Lisinopril on Serum Blood Urea Nitrogen (BUN) and Creatinine. Superscript (a) indicates a significant increase in when compared with control at P<0.05. Superscript (b) indicates significant increase when compared with the hypertensive group at P<0.05. **Abbreviations**: (HYPR) Hypertensive; (L-ARG) L-Arginine; (LISP) Lisinopril.

Figure 3

See image above for figure legend.

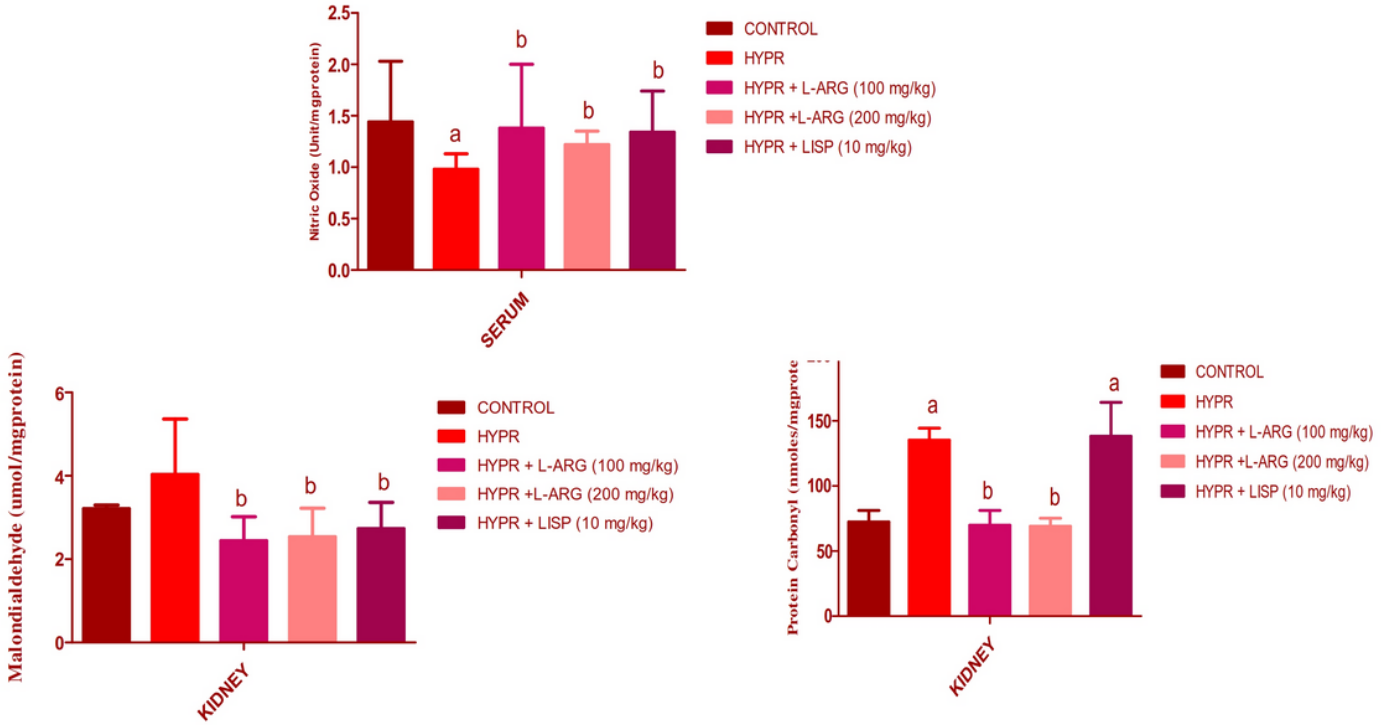

Fig. 4 Effects of L-Arginine on renal markers of oxidative stress and serum nitric oxide. Superscript (a) indicates a significant increase in when compared with control at P<0.05. Superscript (b) indicates significant increase when compared with the hypertensive group at P<0.05. **Abbreviations**: (HYPR)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

Figure 4

See image above for figure legend.

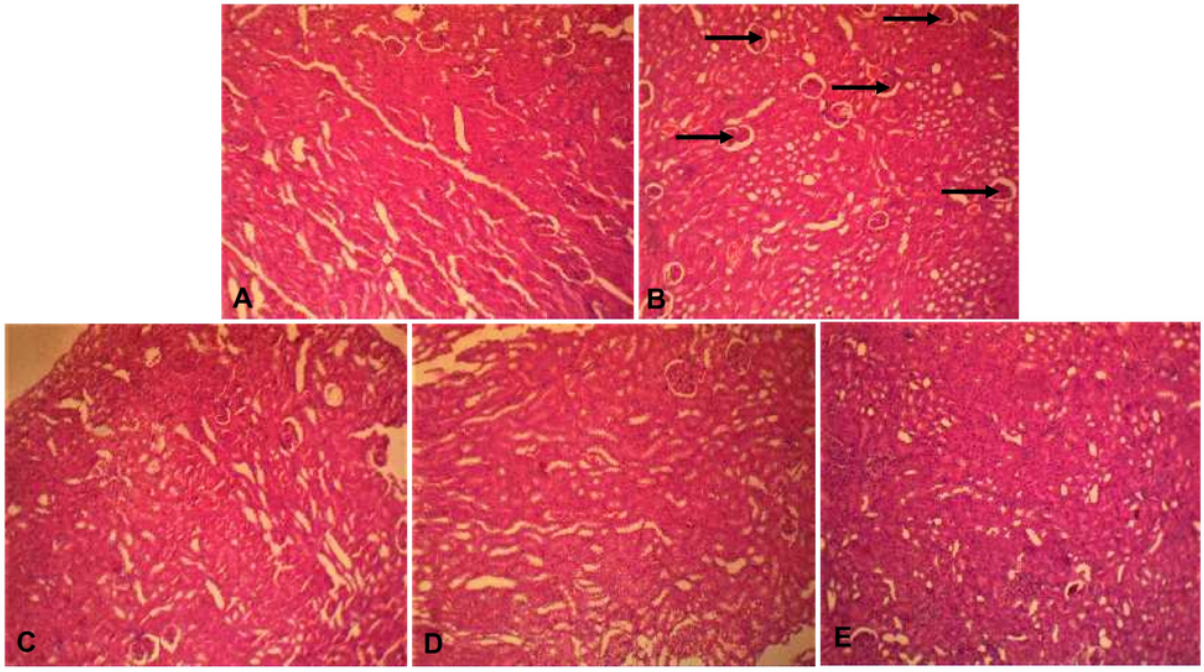

Fig. 5 The histology of the kidney. Group A (Control), Group B (HYPR (NaF; 300 ppm), Group C (HYPR + L-ARG 100 mg/kg), Group D (HYPR + L-ARG 200 mg/kg) & Group E (HYPR + LISP 10 mg/kg). Slides stained with Heamtoxylin and Eosin (H&E). (Magnification x 100). **Abbreviations:** (HYPR) Hypertensive; (L-ARG) L-Arginine; (LISP) Lisinopril.

Figure 5

See image above for figure legend.

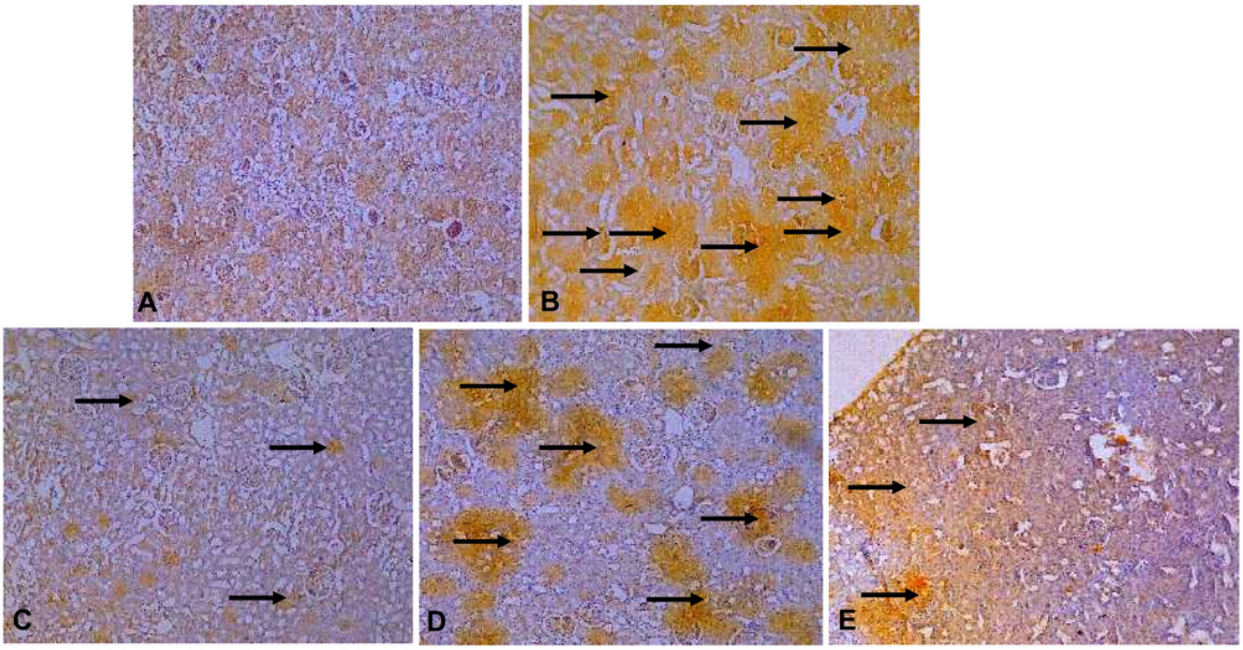

Fig. 6 The immunohistochemistry of renal mineralocorticoid receptor (MCR). Group A (Control), Group B (HYPR (NaF; 300 ppm), Group C (HYPR + L-ARG 100 mg/kg), Group D (HYPR + L-ARG 200 mg/kg) & Group E (HYPR + LISP 10 mg/kg). Slides stained with high definition Heamtoxylin. (Magnification x 100). **Abbreviations:** (HYPR) Hypertensive; (L-ARG) L-Arginine; (LISP) Lisinopril.

Figure 6

See image above for figure legend.

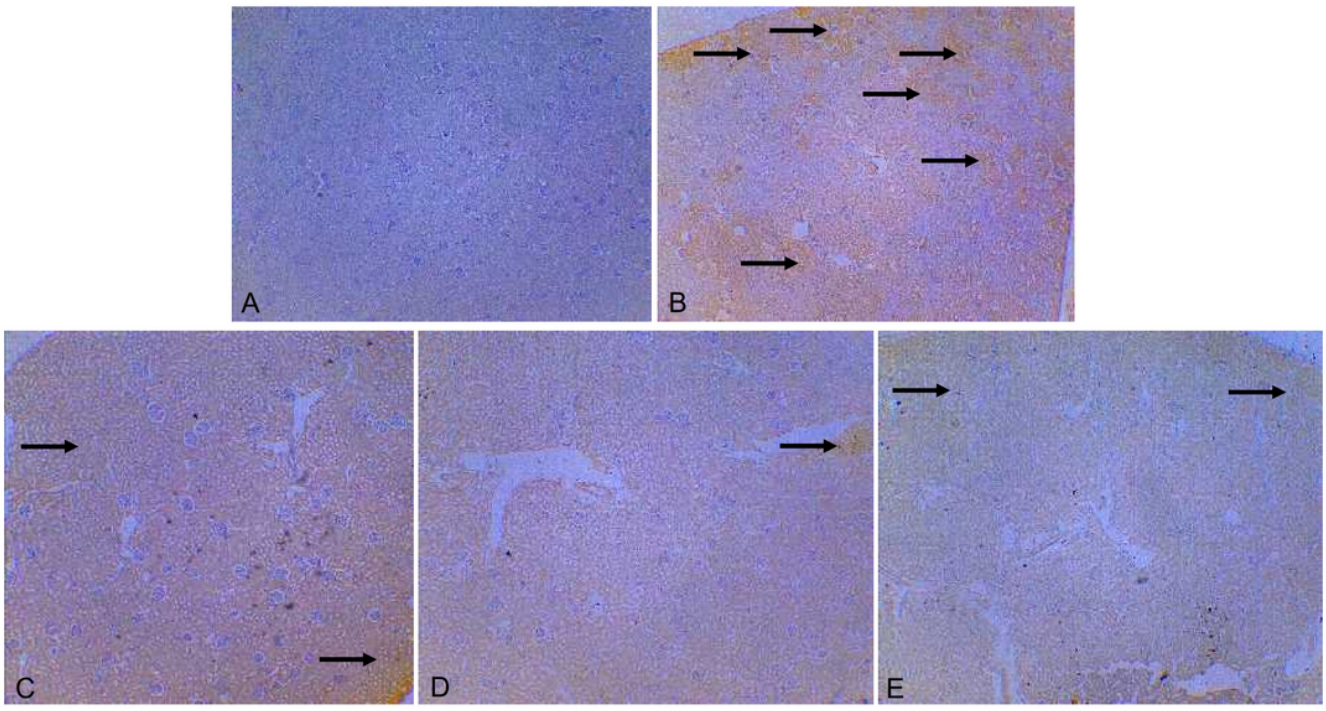

Fig. 7 The immunohistochemistry of renal angiotensin converting enzyme (ACE). Group A (Control), Group B (HYPR (NaF; 300 ppm), Group C (HYPR + L-ARG 100 mg/kg), Group D (HYPR + L-ARG 200 mg/kg) & Group E (HYPR + LISP 10 mg/kg). Slides stained with high definition Heamtoxylin. (Magnification x 100). **Abbreviations:** (HYPR) Hypertensive; (L-ARG) L-Arginine; (LISP) Lisinopril.

Figure 7

See image above for figure legend.