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ORIGINAL ARTICLE

# Cardioprotective and renoprotective effects of melatonin and vitamin E on fluoride-induced hypertension and renal dysfunction in rats

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## Abstract

Fluoride is an important toxicological and environmental toxicant that is implicated in diverse cardiorenal system dysfunctions via the induction of oxidative stress. The present study aims at evaluating the cardioprotective and renoprotective effects of melatonin and vitamin E on fluoride toxicity on biomarkers of oxidative stress, clinical pathology, and their molecular mechanism of action. Apparently healthy male rats of the Wistar strain ($n=50$; $160 \pm 7.5$ g), were randomly distributed into five groups of ten animals per group as follows: Control, sodium fluoride (NaF, 25 mg/kg), NaF and melatonin 20 mg/kg i.p.; NaF and vitamin E 50 mg/kg p/o, NaF plus melatonin and vitamin E administered orally. NaF and melatonin were administered for fifteen consecutive days, whereas vitamin E was administered every 72 h. Blood pressure parameters, oxidative stress biomarkers, electrocardiography, histopathology, and immunohistochemical staining were performed. From this study, NaF intoxication provoked reduction in renal and cardiac systemic antioxidants, alterations of haemodynamic and electrocardiographic parameters, heightened blood urea nitrogen (BUN), creatinine, angiotensin converting enzyme, angiotensin 2 type 1 receptor, kidney injury molecule 1, interleukin 1 beta in the renal tissues, cardiac troponin, and nuclear kappa beta. However, the administration of either melatonin or vitamin E, and its combination mitigated high blood pressure, normalized electrocardiographic changes, abrogated biomarkers of oxidative stress, improved renal function, and attenuated inflammation. The combination of melatonin and vitamin E effectively mitigated cardiovascular and renal toxicities associated with fluoride intoxication through the prevention of cardio-renal dysfunction, oxidative stress, and inflammatory processes.

**Keywords** Fluoride · Hypertension · Melatonin · Rat · Vitamin E

## Introduction

Sodium fluoride (NaF) is an inorganic compound of fluoride that is highly available orally, readily absorbed, and is estimated to be 2 to 5 times more available than other environmental and dietary forms of fluoride (Plumlee 2004). Fluoride is one of the most important toxicological and environmental hazards globally because of its ability to cause fluorosis in humans and livestock (Jha et al. 2013; Kashyap et al. 2021). Paradoxically, dietary fluoride is sine qua non to life (Zhang et al. 2013). In humans, fluorosis commonly manifests following excessive dietary consumption of fluoridated compounds, whereas poisoning of livestock with fluorides, more often than not, is caused by the ingestion of forages contaminated by industrial pollutants or volcanic emissions (Thompson 2018; Cowan and Blakley 2016). Furthermore, toxicity due to excessive fluoride exposure can occur from the use of fluoride containing household products such as dentifrices, special dyes, pesticides, ceramic polishing, and drinking of fluoride-enhanced water (Abdollahi and Momen-Heravi 2014). The wide application of fluoride as anticariogenic agent for the prevention of dental caries may further predispose humans to systemic toxicity (Azab et al. 2018). Hepato-renal, neuronal, and testicular toxicities associated with sodium fluoride intoxications have been documented (Caglayan et al. 2021; Ajibade et al. 2022; Varışlı et al. 2022; Abd-Allah and El-Rahman 2022; Das et al. 2023; Xu et al. 2023). Similarly,

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cardiovascular derangement has been positively correlated with sodium fluoride toxicities from our laboratory and elsewhere (Sharma et al. 2023; Labib et al. 2022; Zhang et al. 2022; Oyagbemi et al. 2017, 2018).

Fluoride reportedly causes diverse physiological alterations in mammalian systems via modulation of enzyme activity and antioxidant defenses, induction of apoptosis, release of cytochrome c from mitochondria, and heightened inflammatory processes (Guth et al. 2020). Alterations of proteins and inhibition of enzyme activities are other important mechanisms involved in the mediation of fluoride toxicities (Mendoza-Schulz et al. 2009). For instance, fluoride potently and specifically inhibits cytoplasmic pyrophosphatases, thereby inhibiting cellular protein functioning in vivo (Strunecka and Strunecky 2020). In addition to the inhibition of pyrophosphatases, fluoride exacerbates the generation of reactive oxygen species (ROS) while abating the levels and or activities of glutathione and other systemic antioxidants (Varol et al. 2013; Strunecká et al. 2019; Srivastava and Flora 2020).

The interactions among different antioxidant molecules can be such that the combined effect of two or more antioxidants is much greater than the sum of the effects of each antioxidant; additive (combined effect of two or more antioxidants is equal to the sum of the effects of each administered antioxidants); or antagonistic (the effects of the antioxidants cancel each other out) (Sonam and Guleria 2017). A logical advantage of the combined use of antioxidants is the significant reduction in the dose of each antioxidants required to produce a desired effect, thereby reducing toxicities associated with each of the exogenous antioxidants. Individually, melatonin and vitamin E are potent antioxidants that modulate the pathogenesis of hypertension and several other mammalian diseases (Borghi and Cicero 2017; Dominguez-Rodriguez et al. 2017; Ferlazzo et al. 2020; Ozkalayci et al. 2021). Vitamin E is a lipid-soluble antioxidant that inhibits the production of ROS and has been reported to protect biological membranes from lipid peroxidation (Hong et al. 2004). Furthermore, bioactive forms of vitamin E such as gamma-tocopherol have been reported to positively modulate cardiovascular system functioning (Rizvi et al. 2014).

A large number of drugs are available for the management of cardiovascular and renal dysfunctions but the use of these drugs is sometimes limited by severe side effects. Therefore, there is a need for the continuous search of safe and effective alternatives, or in the very least, biomolecules that may be used as supplements in the management of cardiac and renal diseases. Although melatonin and vitamin E have been well documented as modulators of cardiovascular and renal systems functioning, to our knowledge, no reports are currently available on the combined effects of the two antioxidants on fluoride-associated cardiovascular/ renal toxicities. Therefore, we sought to assess the mechanistic effects of melatonin and vitamin E alone and in combination on sodium fluoride (NaF)-associated derangements of the cardiorenal systems functioning. The prominent roles of oxidative stress in the pathogenesis of NaF-induced toxicities of the cardiovascular and renal systems made us to posit that melatonin-vitamin E will counteract NaF toxicities and provide new insights into the development of safe alternatives for the prevention and/or management of such diseases as hypertension and renal failure associated with fluoride toxicity in humans and animals.

## Materials and methods

### Experimental animals

Apparently, healthy 8 weeks old male rats of the Wistar strain ($n = 50$; $160 \pm 7.5$ g) were randomly distributed without prejudice to size or weight into five groups of ten animals per group as follows: Control, sodium fluoride (NaF, 25 mg/kg), NaF and melatonin 20 mg/kg i.p.; NaF and vitamin E 50 mg/kg p/o, NaF plus melatonin and vitamin E administered orally. NaF and melatonin were administered for fifteen consecutive days, whereas vitamin E was administered every 72 h. The doses of NaF, melatonin, and vitamin E were used guided by earlier reports of Shanmugam et al. (2018), Ajibade et al. (2017), and Ajibade et al. (2021), respectively. The rats were kept at room temperature (27 °C) and 12-h light/dark cycle. Optimum feed from Ladokun Feeds Nigeria Ltd., Ibadan, Nigeria, and water were made available to the rats which were kept in very clean cages throughout the experimental period of fifteen consecutive days. Male rats were used for the study as estrous cycle of female might interfere with some clinical parameters. Ethical clearance was obtained from the University of Ibadan Animal Care and use research ethics committee (ACUREC) with approval code UIACUREC/ 19/124 assigned.

### Chemicals

The chemicals used in this study were of analytical grade. They include melatonin (AK Scientific, CA, USA), sodium fluoride (NaF) (Molychem, Mumbai, India), trichloro aceticacid, anti-angiotensin converting enzyme1 polyclonal antibody (E-AB-16159: 1:500), angiotensin 2 type 1 receptor (ATR1) polyclonal antibody (E-AB-18016: 1:500), kidney injury molecule 1 (Kim-1) (ab78494: 100), interleukin 1 beta (IL-$\beta$) (E-AB-52153: 1:500), nuclear kappa beta (NF-κB) (E-AB-32232: 1:500), and cardiac troponin (TNNC1) polyclonal antibody (E-AB-18400: 1:50) (Elabscience Biotechnology Inc, Houston, TX, USA); vitamin E and O-dianisidine (Sigma Aldrich, St. Louis, Missouri, USA).

### Plethysmography and electrocardiography

The primary haemodynamic parameters were recorded in conscious carefully restrained rats placed on a warm platform using a plethysmograph (Kent Scientific, USA). The plethysmograph employs the principle of indirect blood pressure measurement. The rats were trained for 30 min and allowed to acclimatize to the conditions of the blood pressure monitor before the haemodynamic parameters were recorded in the most quiescent state of the animals. An average of 30 most consistent readings was recorded for each rat. The electrocardiographic parameters were obtained in xylazine/ketamine anaesthetized rats using an electrocardiograph (EDAN VE-1010, China).

### Preparation of tissues and serum for biochemical assays

The hearts and kidneys were carefully excised and immediately placed on ice to retard enzyme degradation. Thereafter, the tissues were homogenized in potassium phosphate buffer (0.1 M, pH 7.4) and centrifuged at 12,000 g for 15 min to obtain the post-mitochondria fraction (PMF) of the cardiac and renal tissues. The PMFs were persevered in a refrigerator in preparation for biochemical analyses.

Three milliliters of blood were collected from the retroorbital venous plexus in anaesthetized rats with the aid of capillary tubes, into nonheparinised haematological bottles. Thereafter, blood samples were kept at room temperature for 40 min to allow for clot formation and obtain the sera as the supernatant. Then, the separated sera were carefully removed with sterile Pasteur pipettes into clean sample bottles and preserves at −20 °C for biochemical assays.

### Biochemical assays

In this study, the markers of antioxidant defense status such as superoxide dismutase, glutathione, and glutathione peroxidase were assayed in cardiac and renal tissues using the PMF as previously described (Misra and Fridovich 1972; Oyagbemi et al. 2019; Jollow et al. 1974; Buetler et al. 1963). Similarly, the markers of oxidative stress, including malondialdehyde (MDA), nitric oxide NO, and hydrogen peroxide $H_2O_2$, as well as protein concentration were assayed according to the methods established by Varshney and Kale (1990), Olaleye et al. (2007), Wolff (1994), and Gornal et al. (1949), respectively. The breakdown of protein metabolism (urea) and creatinine were assayed in the serum using the corresponding biochemical kits in accordance with the protocol provided by the manufacturer.

### Histopathology

The preparation of the tissues for histopathological evaluation was carried out as described by Bancroft and Gamble (Drury et al. 1976), with the heart and kidney carefully dissected, fixed, processed, mounted on glass slides, and stained with haematoxylin and eosin.

### Immunohistochemistry

The immune-localization of angiotensin converting enzyme (ACE), angiotensin 2 type 1 receptor (ATR1), kidney injury molecule 1 (Kim-1), interleukin 1 beta (IL-$\beta$), nuclear kappa beta (NF-κB), and cardiac troponin were carried out as described by Oyagbemi et al. (2019). Sections were observed with light microscope (Leica LAS-EZ®) using Leica software application suite version 3.4 equipped with a digital camera. Immunoreactivity was quantified using Image J (FIJI) software as described by Fuhrich et al. (2013).

### Statistical analysis

The statistical analysis in this study was carried out using central tendency measures, mean ± standard deviation. Student's *t* test and analysis of variance (ANOVA) were also carried out to compare the statistical difference between means of the experimental groups. Graph Pad Prism version 9.0 was used for the plotting of graphs. Values of probability less than 0.05 were considered statistically significant.

## Results

### Markers of oxidative stress

A significant increase ($P < 0.05$) was recorded in the markers of oxidative stress (hydrogen peroxide and malondialdehyde) in the cardiac and renal tissues of rats following sodium fluoride exposure. Rats administered with melatonin and vitamin E had lesser levels of oxidative stress markers relative to the sodium fluoride treated groups. The combination of melatonin and vitamin E caused a significantly lowered reduction ($P < 0.05$) of oxidative stress markers compared with the individual antioxidants. Moreover, protein carbonyl was elevated following fluoride exposure when compared with rats treated with melatonin and vitamin E.

The systemic antioxidants glutathione peroxidase (GPx), glutathione (GSH), glutathione S-transferase (GST), and superoxide dismutase (SOD) were significantly abated for sodium fluoride-treated group relative to rats administered antioxidants. Higher levels of the antioxidants GPx, GST,

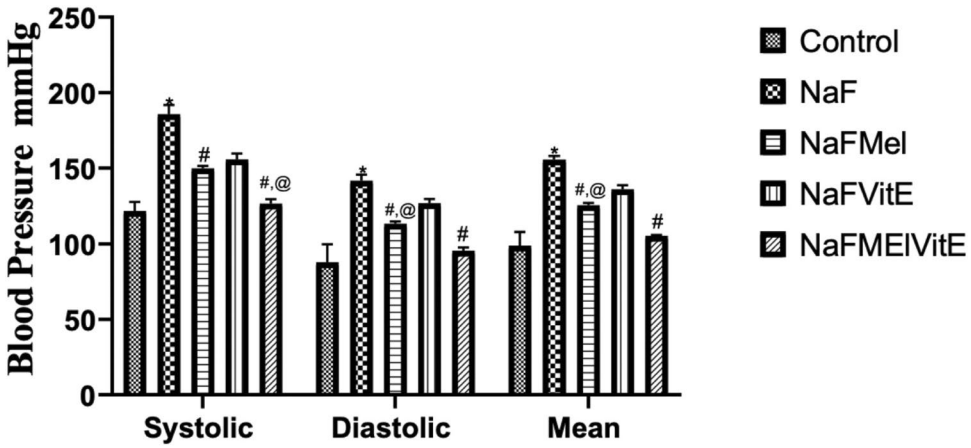


**Fig. 1** Blood pressure parameters of rats exposed to sodium fluoride (NaF) and treated with melatonin (Mel) and vitamin E, alone and in combination. Asterisk (*) indicates significant increase ($P < 0.05$) compared with control; Number sign (#) indicates significant decrease ($P < 0.05$) compared with NaF; Commercial at (@) indicates significant difference ($P < 0.05$) compared with NaFMelVitE

and SOD were seen in rats administered both melatonin and vitamin E compared with rats administered either of the antioxidants.

## Blood pressure and electrocardiogram

The arterial blood pressure (Fig. 1), flow rate (Fig. 2A), as well as heart rate (Fig. 2B) of rats administered sodium fluoride considerably exceeded those recorded for antioxidant-treated rats. Conversely, the electrocardiographic parameters PR interval, QRS complex, and QT interval were significantly shortened in rats administered sodium fluoride relative to the control and the antioxidants-treated rats (Fig. 2C).

## Markers of renal function

The markers of renal function assayed in the serum, in this study (BUN and creatinine) (Fig. 3A, B, respectively), were significantly elevated in the sodium fluoride administered group compared with the control and antioxidant groups of rats.

## Histopathology

Histopathological evaluation revealed necrosis of tubular epithelial cells and peritubular inflammation in the renal tissues, as well as multiple foci of myofiber degeneration in the cardiac tissues of rats administered sodium fluoride, but these lesions were not seen in the tissues of rats administered melatonin (Figs. 4 and 5).

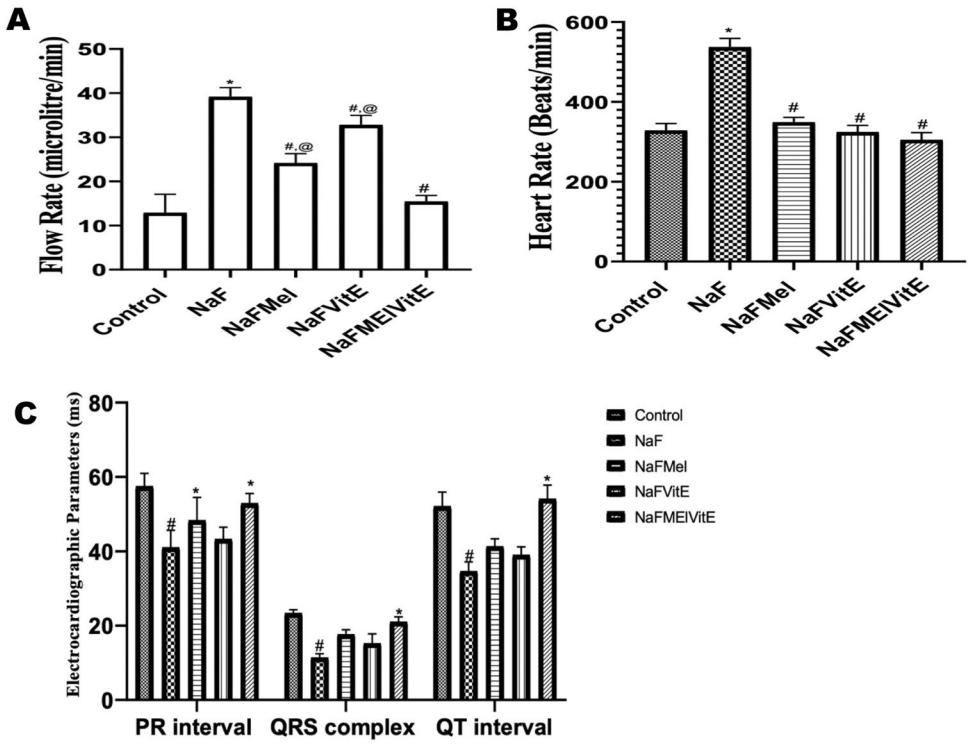


**Fig. 2** Rates of blood flow, heart rate, and electrocardiographic parameters of rats exposed to sodium fluoride (NaF) and treated with melatonin (Mel) and vitamin E, alone and in combination. Asterisk (*) indicates significant increase ($P < 0.05$) compared with control; Number sign (#) indicates significant decrease ($P < 0.05$) compared with NaF; Commercial at (@) indicates significant difference ($P < 0.05$) compared with NaFMelVitE

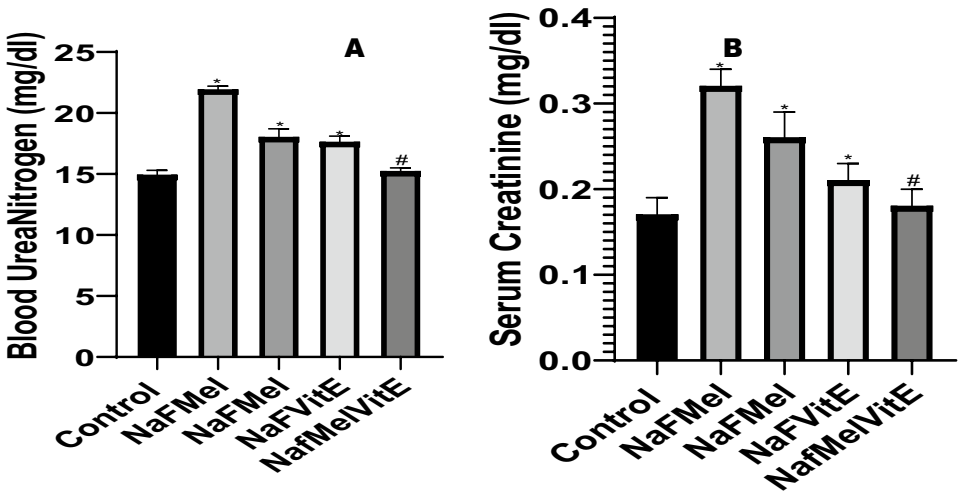


**Fig. 3** Serum blood urea nitrogen (BUN) and creatinine of rats exposed to sodium fluoride (NaF) and treated with melatonin (Mel) and vitamin E, alone and in combination. Asterisk (*) indicates significant increase ($P < 0.05$) compared with control; Number sign (#) indicates significant decrease ($P < 0.05$) compared with NaF

## Immunohistochemical analysis

Immunohistochemical analysis revealed greater expressions of ACE, ATR1, kidney injury molecule 1, and interleukin 1 beta in the renal tissues of rats administered sodium fluoride, compared with the rats treated with melatonin alone or a combination of melatonin and vitamin E (Figs. 6, 7, 8, and 9), whereas greater expressions of cardiac troponin and

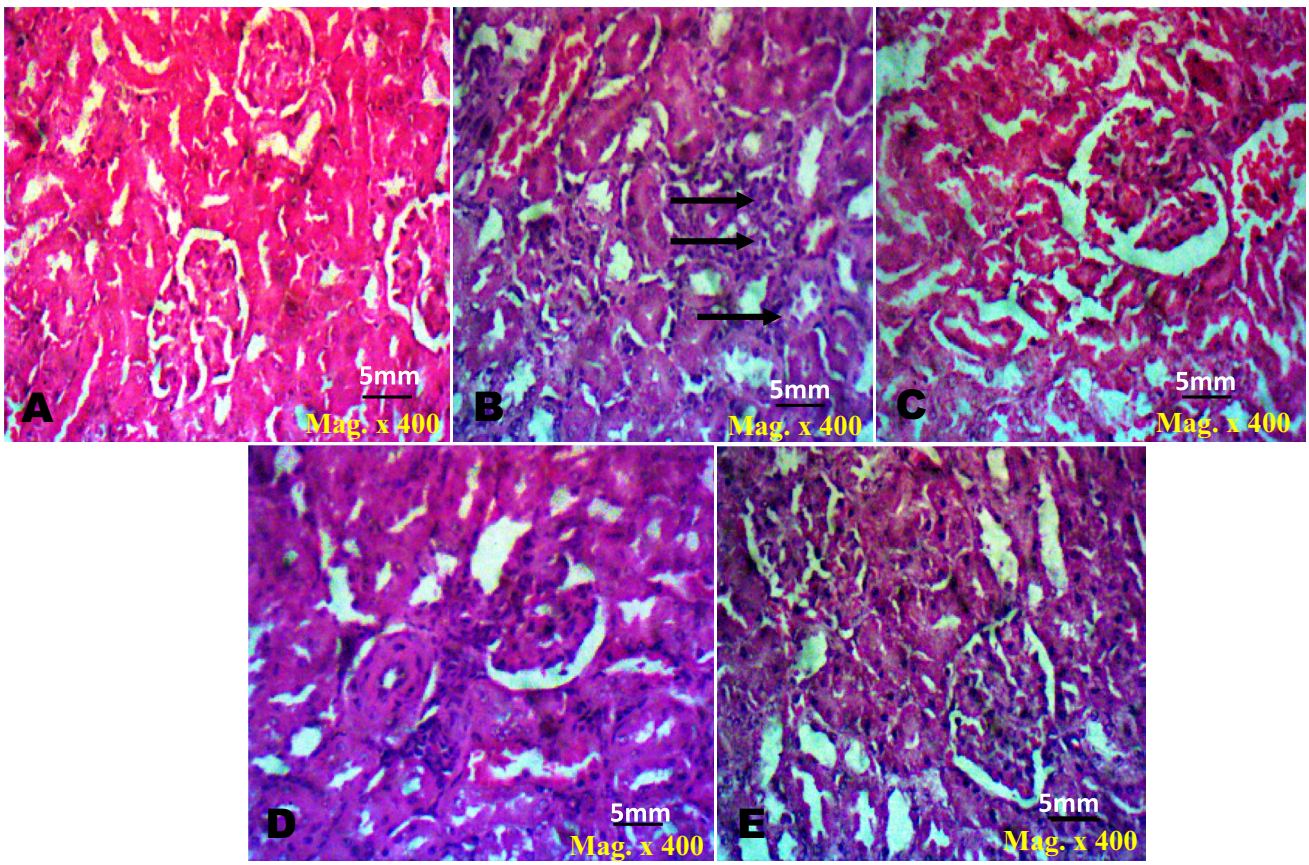


**Fig. 4** Renal tissues of rats administered melatonin and vitamin E in sodium fluoride exposed rats. **A** Control shows no visible lesion. **B** Sodium fluoride shows necrosis of tubular epithelial cells and peritubular inflammation (black arrows). **C** Sodium fluoride and melatonin show mild atrophy of tubular cells. **D** Sodium fluoride and vitamin E shows mild peri-glomerular and tubular inflammation. **E** Sodium fluoride, melatonin, and vitamin E show no visible lesions. H & E. Mag × 400

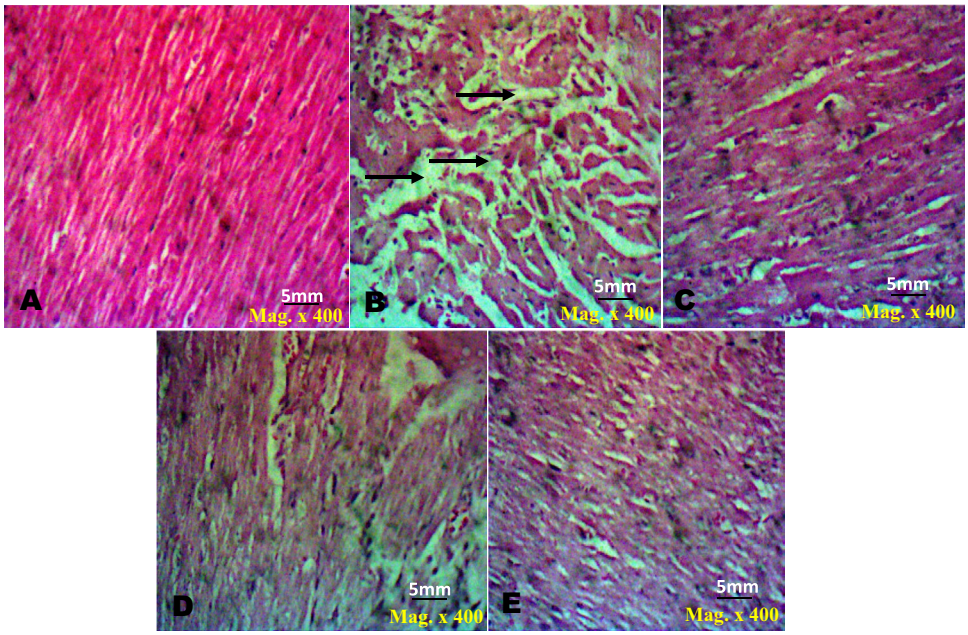


**Fig. 5** Cardiac tissues of rats administered melatonin and vitamin E in sodium fluoride exposed rats. **A** (Control) shows no visible lesion. **B** (Sodium fluoride) shows multiple foci of myofibre degeneration (black arrows). **C** (Sodium fluoride and melatonin) shows no visible lesion. **D** (Sodium fluoride and vitamin E) shows visible lesion. **E** (Sodium fluoride, melatonin and vitamin E) shows no visible lesions. H & E. Mag × 400

nuclear kappa beta (NF-κB) were seen in the cardiac tissues of rats exposed to sodium fluoride without antioxidant treatment, relative to those administered melatonin alone or combinations of melatonin and vitamin E (Figs. 10 and 11).

## Discussion

The heart and kidney are two of the most susceptible soft tissues to fluoride toxicity, with significant alterations

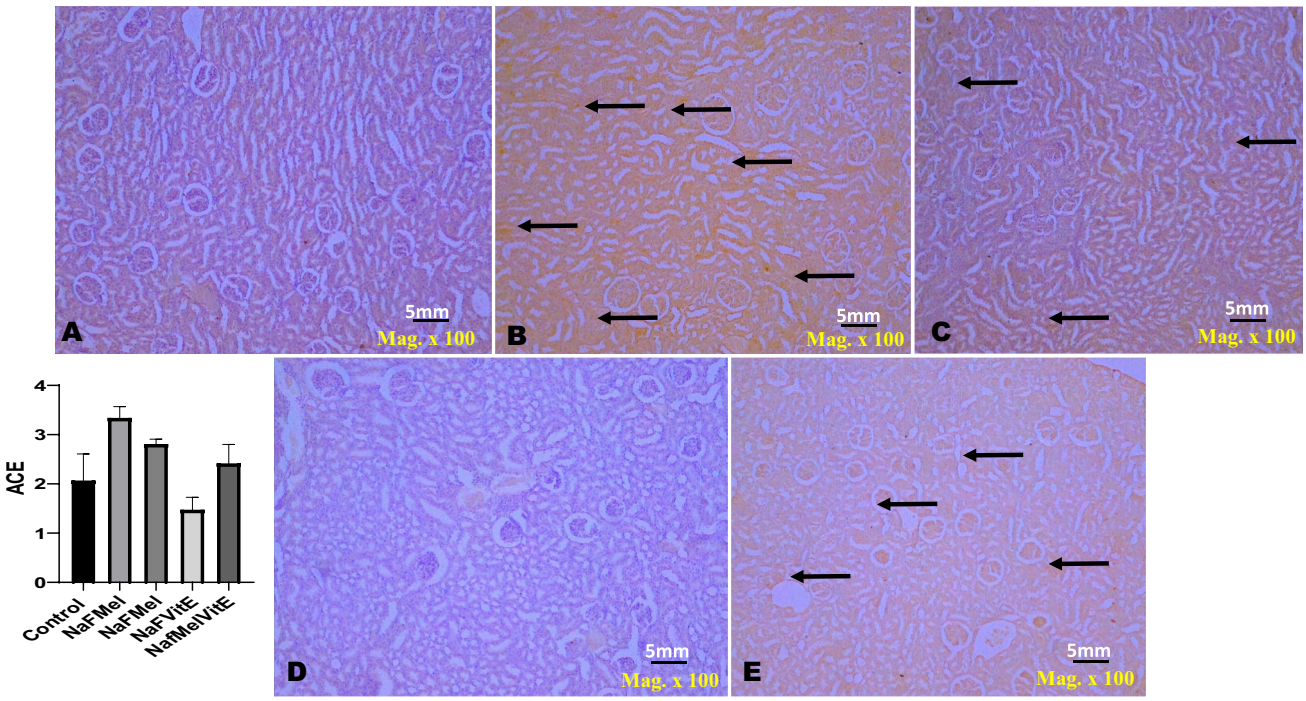


**Fig. 6** The immunohistochemistry of renal angiotensin converting enzyme (ACE). Group A (control), group B (sodium fluoride; 600 ppm), group C (sodium fluoride; 600 ppm + melatonin 50 mg/kg), group D (sodium fluoride; 600 ppm + vitamin E 50 mg/kg), group E (sodium fluoride; 600 ppm + melatonin + vitamin E). The arrows in slides **B**, **C**, and **E** indicate the expression of ACE which is highest in slide **B**. The slides are stained only with haematoxylin. Magnification at × 100

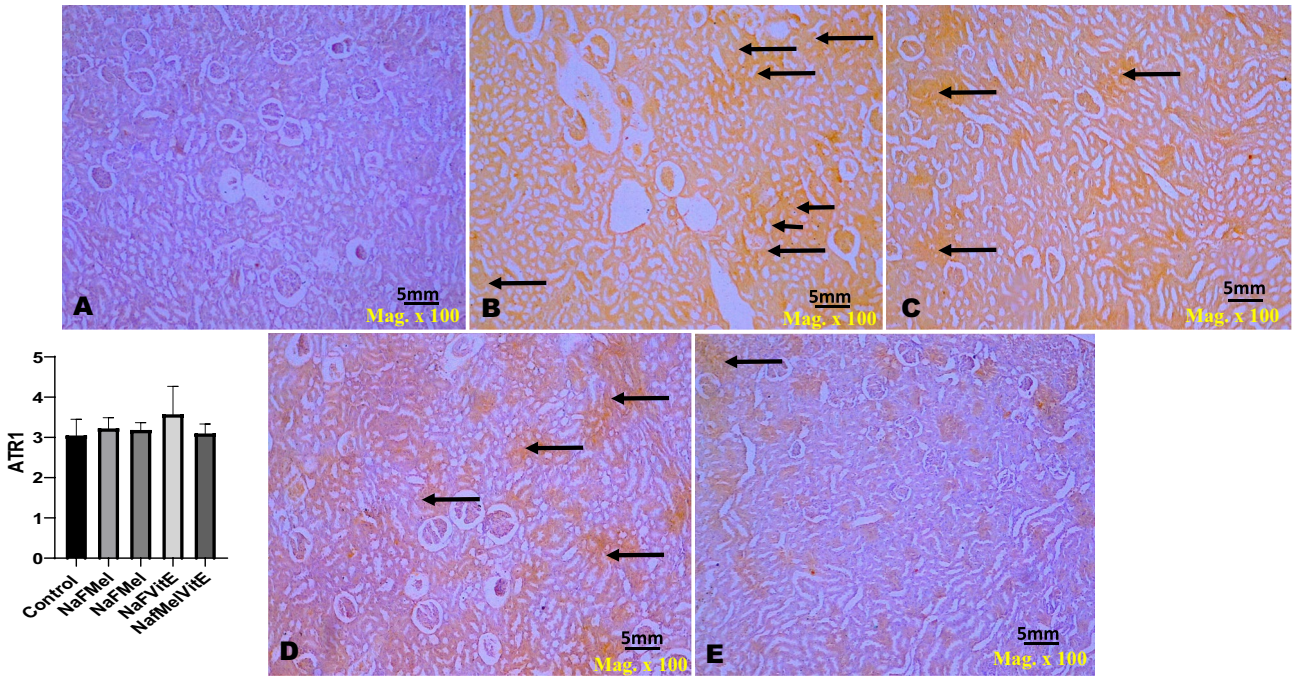


**Fig. 7** The immunohistochemistry of renal angiotensin 2 type I receptor (ATR1). Group A (control), group B (sodium fluoride; 600 ppm), group C (sodium fluoride; 600 ppm + melatonin 50 mg/kg), group D (sodium fluoride; 600 ppm + vitamin E 50 mg/kg), group E (sodium fluoride; 600 ppm + melatonin + vitamin E). The arrows in slides **B**, **C**, **D**, and **E** indicate the levels of expression of ATR 1 which is highest in slide **B**. The slides are stained only with haematoxylin. Magnification at × 100

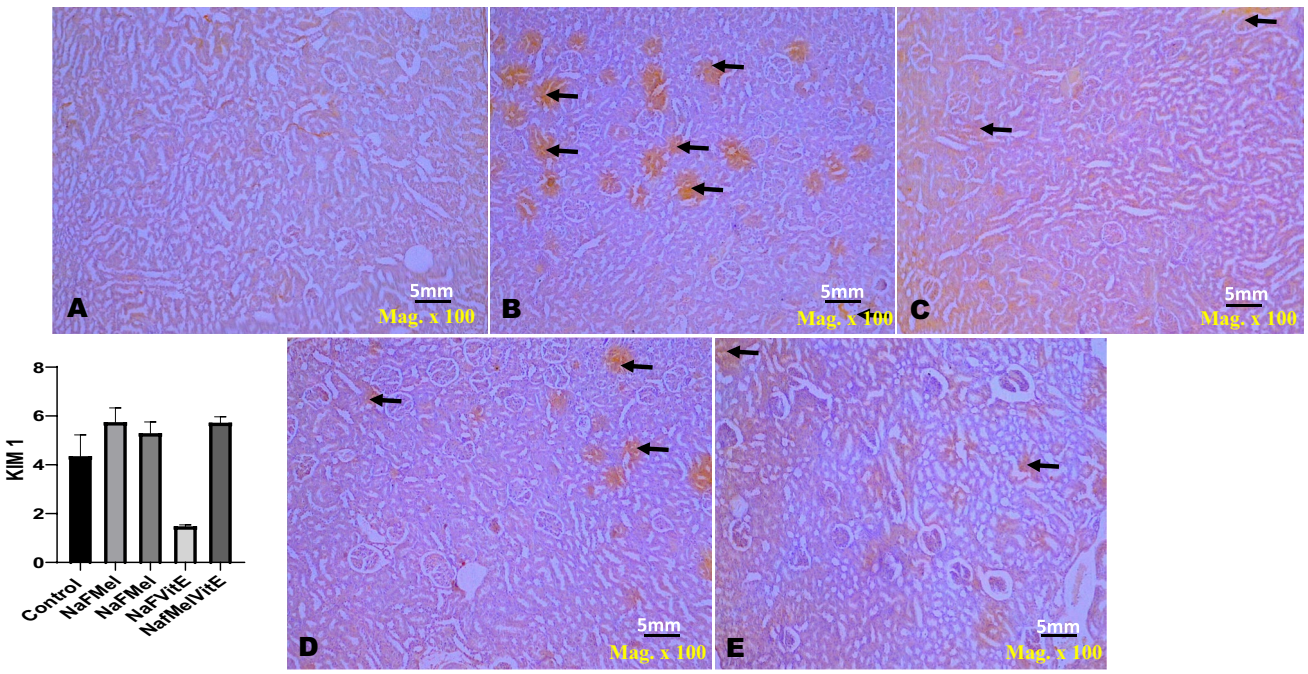


**Fig. 8** The immunohistochemistry of renal kidney injury molecule 1 (Kim-1). Group A (control), group B (sodium fluoride; 600 ppm), group C (sodium fluoride; 600 ppm + melatonin 50 mg/kg), group D (sodium fluoride; 600 ppm + vitamin E 50 mg/kg), group E (sodium fluoride; 600 ppm + melatonin + vitamin E). The arrows in slides **B**, **C**, **D**, and **E** indicate the expression of Kim-1, which is highest in slide **B**. The slides are stained only with haematoxylin. Magnification at × 100

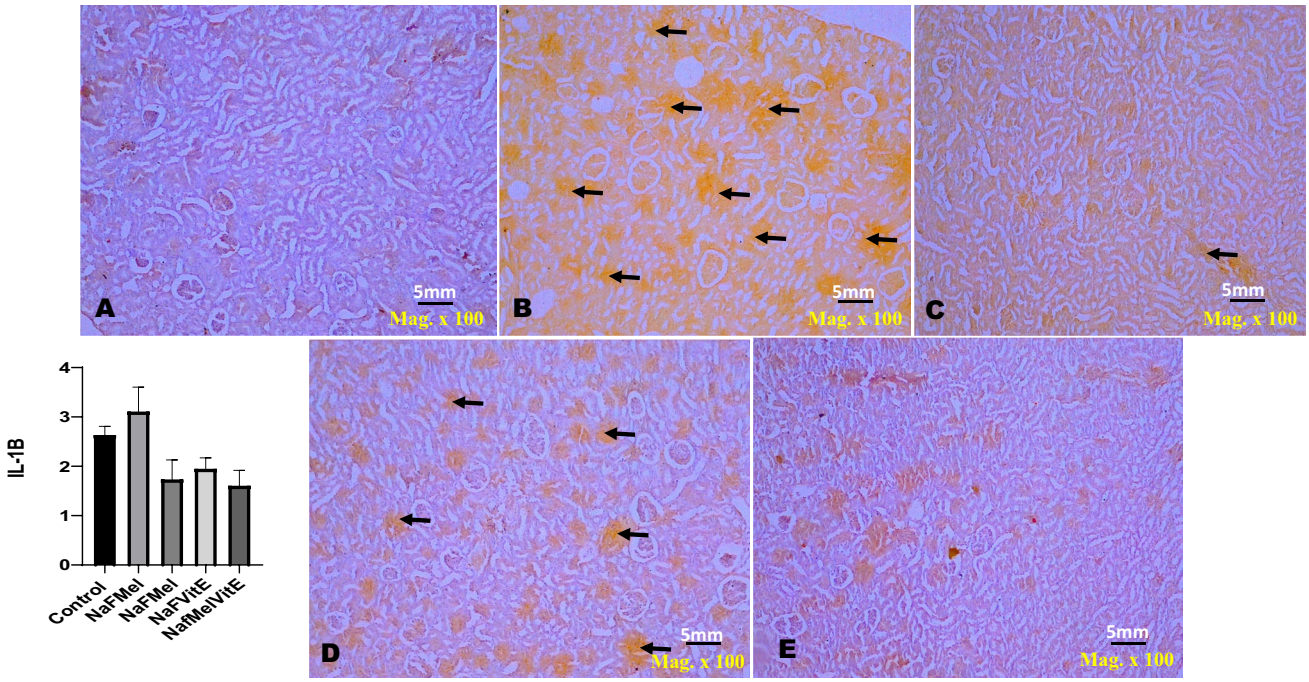


**Fig. 9** The immunohistochemistry of interleukin 1 beta (IL-$\beta$). Group A (control); B (sodium fluoride); C (sodium fluoride and melatonin); D (sodium fluoride and vitamin E); E (sodium fluoride, melatonin, and vitamin E). The arrows in slides **B**, **C** and **D** indicate the levels of expression of IL-$\beta$ which is highest in slide **B**. The slides are stained only with haematoxylin. Magnification × 100

of normal cardiovascular and renal systems functioning reported in human and animal models (Jha et al. 2011). In this study, the exposure to sodium fluoride caused severe dysregulation of the cardiovascular and renal systems, manifested as elevated primary haemodynamic parameters and kidney functional markers, respectively. Fluoride is a

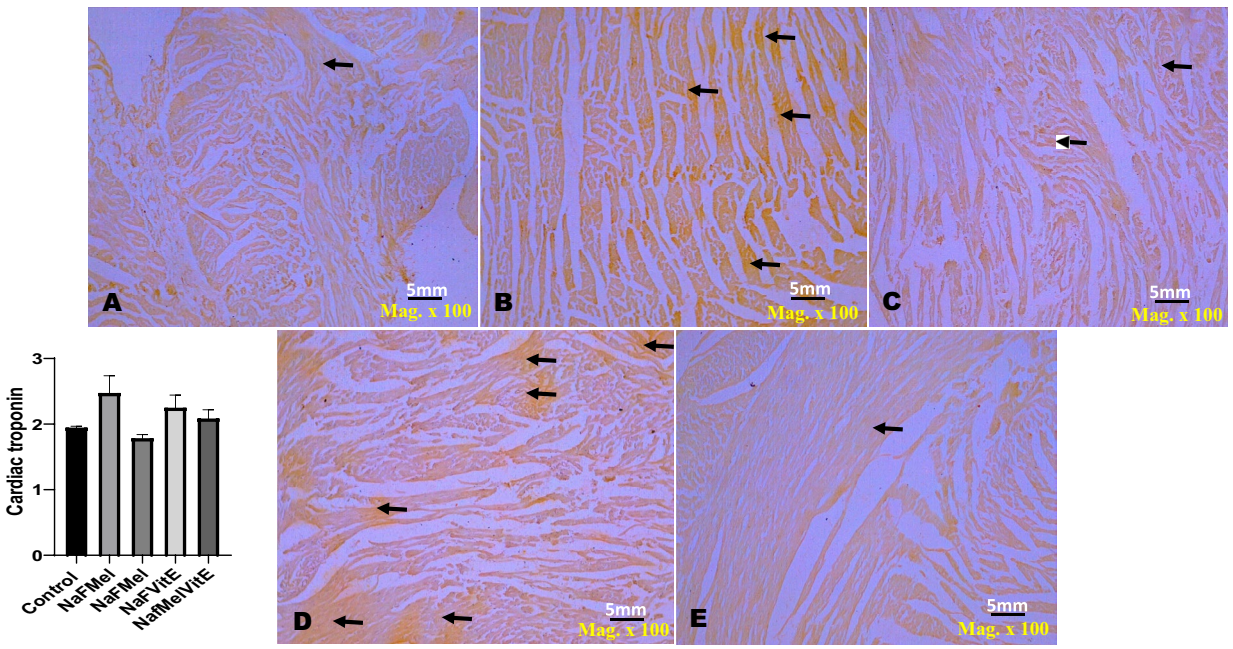


**Fig. 10** The immunohistochemistry of cardiac troponin. Group A (control), group B (sodium fluoride; 600 ppm), group C (sodium fluoride; 600 ppm + melatonin 50 mg/kg), group D (sodium fluoride; 600 ppm + vitamin E 50 mg/kg), group E (sodium fluoride; 600 ppm + melatonin + vitamin E). The arrows in slides **A**, **B**, **C**, **D**, and **E** indicate the levels of expression of cardiac troponin which is highest in slide **B**. The slides are stained only with haematoxylin. Magnification at × 100

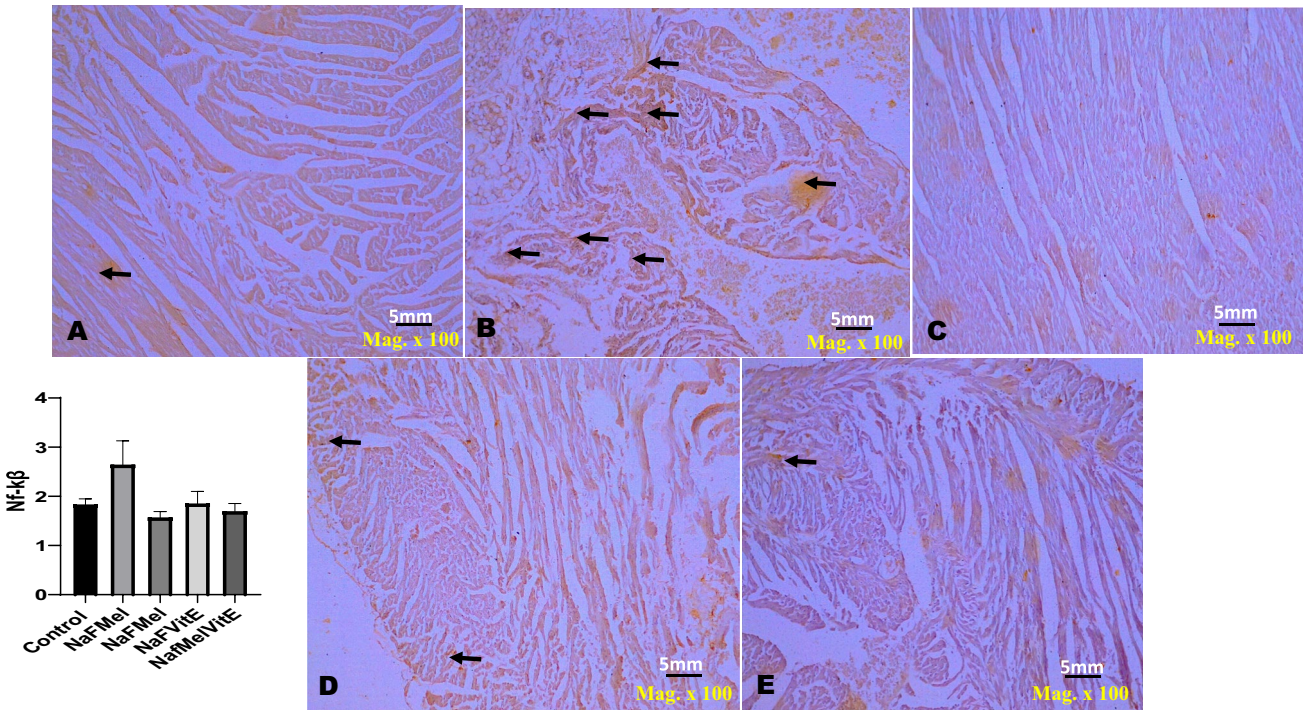


**Fig. 11** The immunohistochemistry of cardiac nuclear kappa beta (Nf-kβ). Group A (control), group B (sodium fluoride; 600 ppm), group C (sodium fluoride; 600 ppm + melatonin 50 mg/kg), group D (sodium fluoride; 600 ppm + vitamin E 50 mg/kg), group E (sodium fluoride; 600 ppm + melatonin + vitamin E). The arrows in slides **A**, **B**, **C**, **D**, and **E** indicate the levels of expression of Nf-kβ which is highest in slide **B**. The slides are stained only with haematoxylin. Magnification at × 100

pro-inflammatory factor that has been reported to mediate significant elevation of blood pressure parameters via the induction of oxidative stress and inflammation in cardiac and renal tissues (Gutowska et al. 2010; Flora et al. 2012). An association between increased fluoride in ground water and increased prevalence of hypertension in adult males has been reported (Amini et al. 2011). However, the administration of the two antioxidants, melatonin, and vitamin E, in this study prevented the development of hypertension and maintained normal levels of serum blood urea nitrogen (BUN) and creatinine in rats. Remarkably, this observation is more pronounced in rats administered both melatonin and vitamin E.

Melatonin is an endogenous neurohormone involved in the control of a number of physiological processes, including circadian rhythms, mood regulation, anxiety, sleep, appetite, immune responses, maintenance of oxidant/antioxidant balance, and cardiovascular functioning (Comai and Gobbi 2014). Melatonin, due to its direct radical scavenging activity, exerts potent antioxidant effects on tissues/organs and antiapoptotic effects on cells (Tamura et al. 2012; Onaolapo et al. 2017). In this study, melatonin alone and in combination with vitamin E significantly decreased the heart rate, as well as the systolic, diastolic, and mean arterial blood pressure, while increasing the duration of the electrocardiographic parameters PR and QT intervals, QRS complex and ST segment, compared with the sodium fluoride administered group. This observation suggests a potent ability of melatonin to mitigate fluoride-induced toxicological effects probably by preventing the development of oxidative stress in the cardiac and renal tissues. In an earlier study, melatonin was reported to inhibit oxidative stress in the brain of rats chronically exposed to NaF with significant decreases reported in the levels of TBARS and ROS while increasing the activities of antioxidant enzymes and GSH content (Jain et al. 2015). In a similar experiment, melatonin, vitamin C, and vitamin E reportedly caused a significant reduction in serum MDA levels of rats exposed to the toxic effects of lead acetate (Aziz et al. 2012). From our results, melatonin, and vitamin E, alone and in combination significantly reduced the biomarkers of oxidative stress—MDA, $H_2O_2$, and PCO, whereas increased levels were recorded for the systemic antioxidants—GPx, GSH, GST, and SOD—relative to rats exposed to the toxicity of NaF without antioxidant treatment. Melatonin together with its metabolites such as cyclic 3-hydroxymelatonin have been reported to scavenge and neutralize a wide array of oxidizing agents and free radicals including singlet oxygen, superoxide anion radical, hydrogen peroxide, nitric oxide, hypochlorous acid, hydroxyl radical, and peroxynitrite anion (Reiter

et al. 2014). In earlier reports, melatonin reportedly exerts indirect antioxidant effects via the potentiation of several enzymatic antioxidants and heightening the efficiency of the mitochondrial electron transport chain (Loren et al. 2017). In addition to its potent antioxidant properties, melatonin exerts highly efficacious anti-inflammatory effects, thereby preventing tissue damage by blocking transcriptional factors of pro-inflammatory cytokines (Guerra and Devesa 2021).

Also, in this study, immunohistochemical evaluation revealed increased expressions of angiotensin 2 type 1 receptor (ATR1), kidney injury molecule 1 (Kim-1), and Interleukin 1 beta (IL-β) in the kidney tissues, whereas expressions of cardiac troponin and nuclear kappa beta were elevated in the cardiac tissues of rats exposed to toxic levels of NaF without antioxidant treatment. In previous studies, fluoride exposure was linked with chronic tubular interstitial nephritis and apoptosis in kidney tissues (Malin et al. 2019), while myocardial necrosis with increased levels of myocardial troponin I, creatine kinase, lactate dehydrogenase, and aspartate transaminase have been reported following experimental fluoride exposure (Panneerselvam et al. 2015). Furthermore, fluoride-induced cell apoptosis in the heart and kidney has been reported to be associated with altered expression of B cell lymphoma/leukemia 2 (Bcl-2), increased cytochrome c, caspase 3p20, and terminal deoxynucleotidyl transferase dUTP nick end labeled positive cells (Agalakova and Gusev 2012). Therefore, the observed attenuation of cardiac troponin and nuclear kappa beta expression, particularly in rats administered both melatonin and vitamin E suggests a mediation of cardioprotective effect via the prevention of inflammatory processes and tissue damage. Melatonin reportedly has a cardioprotective role in a variety of cardiovascular processes via antioxidant, anti-inflammatory, antihypertensive, antithrombotic, and antilipemic effects (Misaka et al. 2019). Interestingly, lowered expressions of cardiac troponin and nuclear factor kappa B (NF-κB) were recorded in the melatonin than the vitamin E administered group; an observation that corroborates an earlier report of Wahab et al. (2000) that melatonin convers a better cardioprotective effect than vitamin E against doxycycline-induced cardiotoxicity. The attenuation of the expression of the markers of tissue damage (cardiac troponin) and inflammation (NF-κB) in the antioxidants treated groups, in this study, is a pointer to the positive modulatory role of melatonin and vitamin E on fluoride-induced cardiovascular dysfunctions. This assertion is further strengthened by the absence of histopathologic lesions in the cardiac tissues of rats administered melatonin and vitamin E. In the kidney tissues, the immunohistochemical expressions of angiotensin converting enzyme (ACE) and kidney injury molecule 1 (Kim-1) were higher in the vitamin E administered group than the melatonin administered group, but the greatest attenuation of immunohistochemical expressions of the two proteins were maximal with the combination of vitamin E and melatonin. In earlier reports, melatonin has been reported to attenuate cisplatin-induced acute kidney injury by suppressing apoptosis and necroptosis in rats (Kim et al. 2019) a report corroborated, in this study, by the absence of histopathological lesions in the heart and kidney tissues of rats administered melatonin alone or in combination with vitamin E.

In conclusion, melatonin and vitamin E effectively mitigate cardiovascular and renal toxicities associated with fluoride exposure through the prevention of oxidative and inflammatory processes as probable mechanisms of action. A major limitation of the study was funding due to the global COVID-19 pandemic that impacted negatively global financial burden. We hope to explore genetic alterations associated with sodium fluoride toxicity during the present post COVID-19 era.

**Author contribution** The authors Ademola Adetokunbo Oyagabemi, Temitayo Olabisi Ajibade, and Temidayo Olutayo Omobowale designed the experiment. The blood pressure was performed by Temitayo Olabisi Ajibade and Temidayo Olutayo Omobowale. Oluwaseun Olanrewaju Esan, Omolola Victoria Awoyomi, and Ademola Adetokunbo Oyagabemi performed the immunohistochemistry and biochemical assays. Moses Olusola Adetona, Temidayo Olutayo Omobowale, Olufunke Eunice Ola-Davies, Adebowale Benard Saba, Adeolu Alex Adedapo, Sanah Malomile Nkadimeng, Lyndy Joy McGaw, Prudence Ngalula Kayoka-Kabongo, Momoh Audu Yakubu, Evaristus Nwulia, and Oluwafemi Omoniyi Oguntibeju supervised, proof-read, and approved the submission.

**Data Availability** Data will be made available on request.

## Compliance with ethical standards

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**Conflict of interest** The authors declare no competing interests.

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