Sodium Fluoride Induces Hypertension and Cardiac Complications through Generation of Reactive Oxygen Species and Activation of Nuclear Factor Kappa Beta

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ABSTRACT: Human exposure to sodium fluoride through its daily usage is almost inevitable. Cardiovascular and renal dysfunction has been associated with fluoride toxicity. Therefore, this study investigated the mechanism of action of sodium fluoride (NaF) induced hypertension and cardiovascular complications Forty male albino rats of an average of 10 rats per group were used. Group A received clean tap water. Toxicity was induced in Group B to D by administering graded doses of NaF through drinking water *ad libitum* for 10 days at 150 ppm, 300 ppm, and 600 ppm concentration respectively. Following administration of NaF, there was significant increase in systolic pressure, diastolic pressure and mean arterial pressure. Markers of oxidative stress; malondialdehyde, hydrogen peroxide, advance oxidation protein products, and protein carbonyl were significantly increased in dose-dependent pattern in the cardiac and renal tissues of rats together with significant decrease in the GST activity in NaF-treated rats compared to the control. Also serum markers of inflammation, cardiac, and renal damage including myeloperoxidase, xanthine oxidase, blood urea nitrogen, creatinine, Lactate dehydrogenase (LDH), and Creatinine kinase myocardial band (CK-MB) significantly increased indicating induction of oxidative stress, renal, and cardiac damage after exposure. Histopathology of the kidney and heart revealed aberrations in the histological architecture in NaF-treated rats. Also, immunohistochemistry showed higher expression of nuclear factor kappa beta (NF-kB) in the cardiac and renal tissues of rats administered NaF. Combining all, these results indicate NaF-induced hypertension through generation of reactive oxygen species and activation of renal and cardiac NF-kB expressions. © 2016 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2016.

Keywords: sodium fluoride; hypertension; cardiovascular complications; ROS; NF-kB

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INTRODUCTION

Fluoride has been widely used in dentistry as prophylactic agent for dental caries and as whitening for the teeth (Leite et al., 2007). However, human exposure to sodium fluoride through its daily usage is almost inevitable. Long-term excessive sodium fluoride (NaF) intake has been reported to cause bone diseases and nonskeletal fluorosis (Song et al., 2014). NaF is known to target the kidneys which serve as the primary organs involved in its excretion and retention (Song et al., 2014). In addition, renal toxicity associated with NaF has been shown to induce apoptosis in the kidney of rats through caspase-mediated pathway and DNA damage (He et al., 2011; Gao et al., 2014). Tubular dysfunction that further resulted in diluted urine, impaired protein reabsorption and increased calcium and phosphate urinary excretion have been documented as one of the mechanisms of action of NaF toxicity (Santoyo-Sanchez et al., 2013) . Banala and Karnati (2015) documented that NaF induced oxidative stress through depletion in levels of various anti-oxidants such as glutathione, superoxide dismutase (SOD), fat soluble vitamins (D and E) with increased levels of lipid peroxidation (LPO). Other mechanisms of action of NaF through which it induces oxidative stress and alters antioxidant defense mechanism have been reported (Feng et al., 2015; Umarani et al., 2015; Samanta et al., 2016). Senescence or normal physiologic aging has also been reported to play a significant role in modulating in the kidney. The microanatomical changes associated with fluoride toxicity have been reported to include decreased number of functional glomeruli due to arteriosclerosis, glomerulosclerosis, and tubular atrophy with interstitial fibrosis together with compensatory hypertrophy of remaining nephrons (Denic et al., 2016). The association between NaF accumulation and negative impact on the cardiovascular system has been reviewed (Janssen et al., 2013).

The incidence of renal failure has increased globally in the last 2 decades (Tonelli et al., 2016). It has been reported that NaF concentrations (>1.5 mg/L) is detrimental to human health (Jha et al., 2013). This therefore justified the need to undertake this study. This study seeks to understand further how NaF induce hypertension and cardiovascular complications together with the underlying mechanism of action.

MATERIALS AND METHODS

Chemicals

1,2-dichloro-4-nitrobenzene (CDNB), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), thiobarbituric acid (TBA), glutathione, hydrogen peroxide, sodium hydroxide, epinephrine, xylenol orange and other chemicals of analytical grade utilized were obtained from Sigma (St. Louis, MO). Normal goat serum, Biotinylated antibody and Horse Radish Peroxidase (HRP) System was purchased from (KPL, Inc., Gaithersburg, MD). NF-kB antibody was purchased from (Bioss Inc. Woburn, MA) while 3, 3′-Diaminobenzidine (DAB) tablets were purchased from (AMRESCO LLC. OH). All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

Experimental Design and Animal Treatment

Forty adult male rats of the Wistar strain weighing approximately 125–175 g were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan and randomly divided into 4 groups of 10 animals each. The rats were maintained on commercial pelletized rats finisher feed and water was provided *ad libitum*. The animals were kept in plastic cages under controlled light cycle (12 h light/12 h dark). Group A received clean tap water. Toxicity was induced in Group B to D by administering graded doses of NaF through drinking water *ad libitum* for 10 days at 150 ppm, 300 ppm, and 600 ppm concentration, respectively.

The animals were humanely cared for according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. During the experiment, ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animal's welfare (PHS, 1996).

Blood Pressure Measurement

The rats were carefully restrained on lateral recumbency on a well-padded platform. Indirect blood pressure parameters (systolic, diastolic and mean blood pressure) were determined without anaesthesia, by tail plethysmography using an electrosphygnomanometer (CODA, Kent Scientific, USA). The average of at least nine readings, taken in the quiescent state, following acclimatization, was recorded per animal.

Serum Preparation and Isolation of Post-Mitochondrial Fraction

About 3 mL of blood was collected from the retro-orbital venous plexus of the animals into plain sample bottles before they were sacrificed by cervical dislocation. The blood was centrifuged at 4000 rpm for 15 min to obtain the serum. The kidney and hearts were harvested on ice, rinsed, and homogenized in aqueous potassium buffer (0.1 M, pH 7.4) and the homogenate centrifuged at 10,000 rpm (4°C) for 10 min to obtain the supernatant fraction.

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Biochemical Assays

The post-mitochondrial fractions of the heart and kidney were used for the estimation of reduced GSH as described by Beutler et al. (1993). Catalase activity was estimated using the method of Claiborne (1985). Glutathione-S-transferase was measured by the method of Habig et al. (1974) and Glutathione peroxidise activity was determined as described by Rotruck et al. (1973). Superoxide dismutase was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 as described by Misra and Fridovich (1972) and with modification from our laboratory (Oyagbemi et al., 2015; Omobowale et al., 2014). The MDA level was calculated as described by Varshney and Kale (1970). Lipid peroxidation in μmol MDA formed/mg protein was computed with a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Hydrogen peroxide generation was estimated as described (Wolff, 1994). Protein concentration was determined by Biuret method as described by Gornal et al. (1949). Nitric Oxide was measured as described by Olaleye et al. (2007).

The sulfhydryl (total thiol) and non-protein thiol (NPT) content was determined as described by Ellman (1959).The activity of xanthine oxidase was determined according to method of Akaike *et al*., (1990). The serum myeloperoxidase activity was determined according to the method of Xia and Zweier (1997). The manufacturer's protocol for the determination of serum creatinine, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), creatinine kinase myocardial band (CKMB), and aspartate aminotransferase (AST) was followed. All the kits for this study were purchased from Randox Ltd, UK.

Histopathology

The whole heart and kidney tissues were fixed in 10% buffered formalin. These tissues were processed and embedded in paraffin wax. Totally, 5–6 μm thick sections were made and stained with haematoxylin and eosin for histopathological examination (Drury et al., 1976).

Immunohistochemistry of Cardiac and Renal Nuclear Factor Kappa Beta (NF-kB)

Immunohistochemistry of paraffin embedded tissue of the kidney and heart was performed after the tissues were fixed with 10% formalin. The tissues were processed for immunohistochemistry based on the methods described by Todorich et al. (2011) with medication. Briefly, paraffin sections were melted at 60°C in the oven. Dewaxing of the samples in xylene was followed by passage through ethanol of decreasing concentration (100–80%). Peroxidase quenching with 1% H_2O_2/methanol was carried out with subsequent antigen retrieval performed by microwave heating in 0.01 M citrate buffer (pH 6.0) to boil. All the sections were blocked in normal goat serum (10%, HistoMark®, KPL, Gaithersburg MD) and probed with nuclear factor kappa beta (NF-kB) antibody (Bioss, San Diego, CA), 1:200 overnight at room temperature. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 μg/mL) secondary antibody and subsequently, streptavidin peroxidase (Horse Radish Peroxidase- streptavidin) according to manufacturer's protocol (HistoMark®, KPL, Gaithersburg MD).

Reaction product was enhanced with diaminobenzidine (DAB, Amresco®, USA) for 2–3 min and counterstained with high definition hematoxylin (Enzo®, NY), with subsequent dehydration in ethanol. The slides were covered with coverslips and sealed with resinous solution. The immunoreactive positive expression of NF-kB intensive regions were viewed starting from low magnification on each slice then with 100 × magnifications using a photo microscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China). The immune-positive reactions are quantified with Image J software.

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Statistical Analysis

All values are expressed as mean ± standard deviation (SD). The test of significance between two groups was estimated by Student's *t*-test. One way Analysis of Variance (ANOVA) with Tukey's post-hoc test using Graph pad prism 5.0 was also performed with p-values < 0.05 considered statistically significant.

RESULTS

Results from Table I showed significant ($p < 0.05$) increase in the heart and kidney weight in the group exposed to NaF except rats dosed with 300 mg/L NaF compared to the control. Rats exposed to 150 mg/L of NaF had significant decrease in heart/body weight ratio compared to the control. There was also significant decrease in kidney weight and kidney/body ratio in all the groups exposed to NaF when compared to the control. All the groups exposed to NaF showed significant increase in values of systolic blood pressure, diastolic blood pressure, and mean arterial pressure when compared to the control (Table I).

There was significant increase in the renal GST activity of rats in group B (150 mg/L NaF) but significant decrease in the GST activity in rats that received 300 and 600 mg/L NaF (groups C and D) when compared to the control (Table II). There was significant increase in renal CAT activity in the toxicant all the groups (B, C, and D) compared to the control. Similarly, significant increase in the activity of SOD was observed in rats that received 300 and 600 mg/L NaF respectively (Table II). There was significant decrease in cardiac GPx and CAT activities whereas significant increase in SOD and GST activities was obtained in rats administered NaF when compared to the control (Table III). The renal GSH reduced significantly in NaF administered groups as compared to the control. On the other hand; the renal MDA,

TABLE I. The effect of sodium fluoride intoxication on body weights, organ weights, and blood pressure parameters

Parameters	Group A	Group B	Group C	Group D
Heart weight (g)	0.41 ± 0.07	0.44 ± 0.054	0.49 ± 0.053*	0.42 ± 0.14
Heart/body weight (g/g)	0.003 ± 0.0005	0.0028 ± 0.012*	0.0031 ± 0.006	0.0031 ± 0.009
Kidney weight (g)	0.76 ± 0.11	0.88 ± 0.03*	0.92 ± 0.07*	0.90 ± 0.31*
Kidney/body weight (g/g)	0.0064 ± 0.0005	0.0057 ± 0.006*	0.006 ± 0.008*	0.006 ± 0.021*
Systolic blood pressure (mmHg)	124.3 ± 12.64	178.3 ± 20.66*	174.9 ± 25.4*	206.7 ± 5.73*
Diastolic blood pressure (mmHg)	92.41 ± 16.05	129.71 ± 28.5*	122.7 ± 32.79*	178.9 ± 6.24*
Mean arterial pressure (mmHg)	102.66 ± 14.76	145.57 ± 25.69*	139.9 ± 29.67*	187.8 ± 5.67*

Values are presented as mean ± standard deviation ($n = 10$).
*Indicates significant difference at $p < 0.05$.
Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF).

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TABLE II. Renal antioxidant enzymes status in rats following exposure to sodium fluoride (NaF)

Parameter	Group A (Control)	Group B (150 mg/mL)	Group C (300 mg/mL)	Group D (600 mg/mL)
GST	0.019 ± 0.0021	0.026 ± 0.0024*	0.015 ± 0.0015*	0.014 ± 0.0006*
CAT	1.56 ± 0.33	4.58 ± 0.23*	4.66 ± 0.54*	3.58 ± 0.5*
SOD	0.30 ± 0.006	0.30 ± 0.01	0.32 ± 0.003*	0.34 ± 0.017*

Values are presented as mean ± standard deviation ($n = 10$).
Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF), and Group D (600 mg/L of NaF).
*Indicates significant difference at $p < 0.05$. GPx (Glutathione peroxidase; (units/mg protein); GST (Glutathione-S-transferase; mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); CAT (Catalase; mmoleH_2O_2 consumed/min/mg protein); SOD (superoxide dismutase; units/mg protein).

TABLE III. Cardiac antioxidant enzymes status in rats following exposure to sodium fluoride (NaF)

	Group A (Control)	Group B (150 mg/mL)	Group C (300 mg/mL)	Group D (600 mg/mL)
GPx	36.19 ± 3.268	34.17 ± 2.201	32.62 ± 2.1	32.29 ± 1.196*
GST	0.007 ± 0.005	0.008 ± 0.0016	0.012 ± 0.0015*	0.013 ± 0.0018*
CAT	2.85 ± 0.48	1.43 ± 0.15	1.59 ± 0.28*	1.31 ± 0.19*
SOD	0.28 ± 0.025	0.29 ± 0.015	0.29 ± 0.02	0.22 ± 0.01*

Values are presented as mean ± standard deviation ($n = 10$).
Group A (Control), Group B (150 mg/L of NaF), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF).
*Indicates significant difference at $p < 0.05$. GPx (Glutathione peroxidase; (units/mg protein); GST (Glutathione-S-transferase; mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein); CAT (Catalase; mmoleH_2O_2 consumed/min/mg protein); SOD (superoxide dismutase; units/mg protein).

TABLE IV. Renal glutathione content and markers of oxidative stress following exposure to sodium fluoride (NaF)

Parameters	Group A (Control)	Group B (150 mg/mL)	Group C (300 mg/mL)	Group D (600 mg/mL)
GSH	72.50 ± 1.311	70.75 ± 0.979*	69.25 ± 0.913*	68.82 ± 0.4*
MDA	0.24 ± 0.016	0.33 ± 0.009*	0.34 ± 0.038*	0.33 ± 0.017*
TOTAL THIOL	0.03 ± 0.002	0.05 ± 0.008*	0.05 ± 0.004*	0.07 ± 0.002*
NPT	0.1 ± 0.008	0.07 ± 0.003*	0.06 ± 0.002*	0.07 ± 0.004*

Values are presented as mean ± standard deviation ($n = 10$).
Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF), and Group D (600 mg/L of NaF).
*Indicates significant difference at $p < 0.05$. GSH (Reduced Glutathione; micromole/g tissue); MDA (Malondialdehyde; micromole/mg protein); TOTAL THIOL (nmole/mg protein); NPT (nmole/mg protein).

TABLE V. Cardiac glutathione content and markers of oxidative stress following exposure to sodium fluoride (NaF)

Parameters	Group A (Control)	Group B (150 mg/mL)	Group C (300 mg/mL)	Group D (600 mg/mL)
GSH	68.25 ± 1.52	68.08 ± 0.629	67.62 ± 0.322	65.55 ± 0.481*
MDA	0.32 ± 0.035	0.65 ± 0.039*	0.67 ± 0.033	0.71 ± 0.056*
TOTAL THIOL	0.03 ± 0.003	0.03 ± 0.003	0.03 ± 0.001	0.03 ± 0.001
NPT	0.06 ± 0.004	0.07 ± 0.002*	0.06 ± 0.005	0.06 ± 0.003

Values are presented as mean ± standard deviation ($n = 10$).
Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L l of NaF), and Group D (600 mg/L of NaF).
*Indicates significant difference at $p < 0.05$. GSH (Reduced Glutathione; micromole/g tissue); MDA (Malondialdehyde; micromole/mg protein); TOTAL THIOL (nmole/mg protein).
Nonprotein thiol (NPT) (nmole/mg protein).

TT, and NPT increased significantly in the rats administered NaF (Table IV). There were significant decrease in cardiac GSH with concomitant significant increase in the cardiac MDA and NPT content in all the groups exposed to NaF compared to the control (Table V). There was dose-dependent significant increase in serum Creatinine, BUN, LDH, CK-MB MPO and XO in all groups administered with NaF when compared to the control indicating cardiac and renal damage (Table VI).

Our results indicated significant reduction in renal and cardiac NO content in a dose-dependent manner relative to the control (Fig. 1). The renal and cardiac makers of oxidative stress including advanced oxidation protein products (AOPP), hydrogen peroxide (H_2O_2) generated and protein carbonyl (PC) increased significantly in NaF treated rats when compared to the control (Figs. 2–8) respectively.

The ultrastructure of the heart showed infiltration of the myocardial interstistium by inflammatory cells in NaF administered group while the rats in the control group showed no visible lesions (Fig. 9). Figure 10 shows the normal architecture of the kidney with little inflammatory cells in the control group. In contrast rats administered with NaF showed focal peritubular and periglomerular infiltration by inflammatory cells, fusion of the glomeruli with the capsule, necrosis and congestion of vessels. The immunohistochemistry revealed positive immunoreativity with higher expressions of NF-kB in the kidney and hearts of rats exposed to NaF while the control showed lower expressions (Figs. 11 and 12).

DISCUSSION AND CONCLUSION

Several studies have established a close correlation between fluoride intake and renal injury as well as cardiac damage. It is believed that free radical generation with significant alteration in endogenous antioxidant defence system has been suggested to be the mechanism of NaF-induced toxicity (Sarkar et al., 2014).

According to this study, there were dose-dependent significant increases in the blood pressure parameters following exposure to NaF. The result of this study revealed significant increases in systolic pressure, diastolic pressure, mean arterial pressure (MAP), and decrease nitric oxide (NO) levels in rats exposed to graded doses of NaF (150 ppm, 300 ppm and 600 ppm) compared with the control. This study therefore corroborates the study of Amini et al. (2011) who reported on the relationship between fluoride concentrations in ground water and the incidence of high blood pressure in the exposed population. Nitric oxide produced from the vascular endothelium helps to maintain a continuous vasodilator tone that is essential for the regulation of blood flow, blood pressure, platelet aggregation and vasodilation (Hermann et al., 2006). Hence, reduced NO bioavailability has been

TABLE VI. Serum markers of renal and cardiac damage following to sodium fluoride (NaF)

Parameter	Group A (Control)	Group B (150 mg/mL)	Group C (300 mg/mL)	Group D (600 mg/mL)
BUN	33.55 ± 3.89	35.51 ± 2.23	35.52 ± 2.21	135.35 ± 17.30*
CREATININE	13.20 ± 1.55	17.08 ± 1.79*	20.19 ± 3.11*	28.98 ± 3.59*
LDH	701.59 ± 58.36	846.03 ± 87.54	907.94 ± 233.45	1169.32 ± 190.62*
CK-MB	107.31 ± 35.02	206.37 ± 35.02	264.16 ± 19.06*	140.33 ± 11.67
AST	37.00 ± 3.53	57.00 ± 3.53	192.00 ± 3.53*	125.00 ± 17.32*
XO	0.0055 ± 0.0007	0.007 ± 0.0014	0.014 ± 0.002*	0.008 ± 0.0006*
MPO	0.0007 ± 0.00009	0.0013 ± 0.0002*	0.0019 ± 0.0006*	0.0027 ± 0.0005*

Values are presented as mean ± standard deviation ($n = 10$).
Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF).
*Indicates significant difference at $p < 0.05$. BUN (Blood Urea Nitrogen; mmol/l); Creatinine (mmol/l); LDH (U/L); CK-MB (mg/dL); AST (Aspartate aminotransferase; U/L); XO (Xanthine Oxidase; µmole/L); MPO (Myeloperoxidase; µmole/L).

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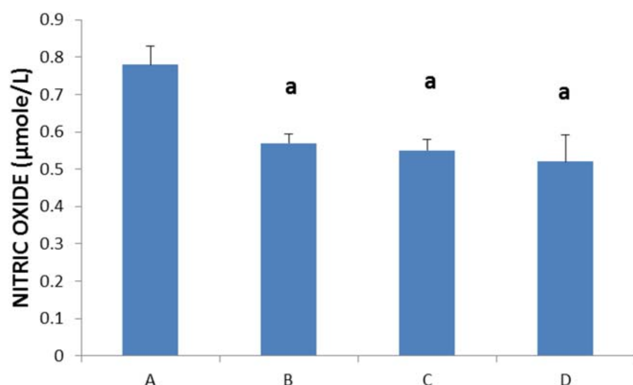

Fig. 1. Effects of sodium fluoride on cardiac nitric oxide level. Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF), and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p < 0.05$, when, B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

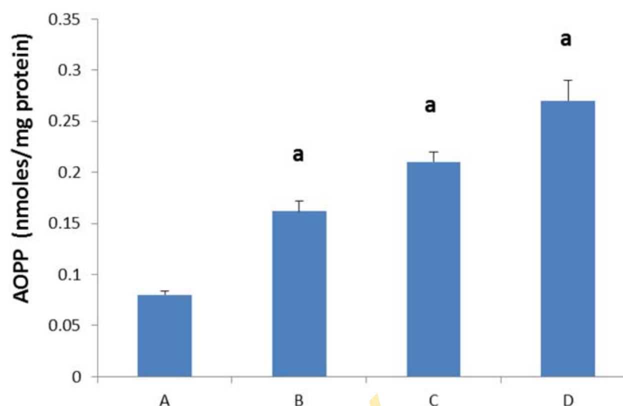

Fig. 3. Cardiac levels of advanced oxidation protein products (AOPPs) following exposure to sodium fluoride. Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p < 0.05$, when, B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

associated with endothelial dysfunction and hypertension (Kelm, 2003; Chalupsky et al., 2015).

Studies have established considerable decrease in level of nitric oxide especially in patients with essential hypertension and renal disease compared with normotensive individuals (Virdis et al., 2015; Wrzosek et al., 2015).

The present study also revealed significant changes in the activities of markers of oxidative stress in the kidney and heart tissues. Also, significant increases in MDA, H_2O_2, AOPPs, protein carbonyl and Non protein thiol (NPT) following exposure to NaF were observed. H_2O_2 can produce cytotoxicity in endothelial cells of different organs. However, the presence of endogenous GSH and antioxidant enzymes such as CAT, GPx and GST has been suggested to be responsible for the elimination of H_2O_2 and other toxic substances (Sharma et al., 2010). Dose-dependent increase in MDA was also observed in the cardiac tissue of animals exposed to NaF. The elevated MDA levels led to the enhanced lipid peroxidation was probably due to the production of superoxide, peroxyl, and hydroxyl radicals (Abdel-Wahhab et al., 2008). Increased peroxidation of membrane lipids is one of principal consequences of oxidative damage produced by NaF exposure (Miltonprabu and Thangapandiyan, 2015; Ameeramja et al., 2016). The cardiac and renal GSH concentrations reduced significantly with the increasing NaF concentration. This clearly indicates an induction of

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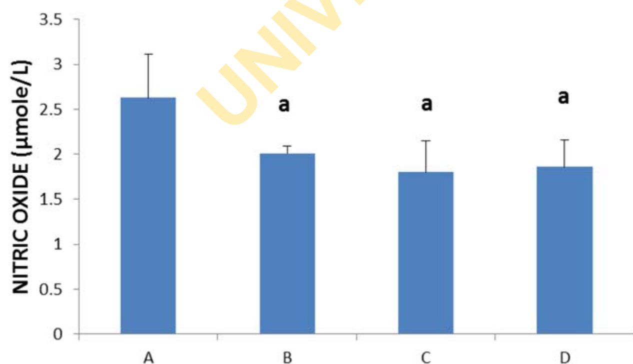

Fig. 2. Effects of sodium fluoride (NaF) on renal nitric oxide (NO) level. Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p<0.05$, when, B, C, and D compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

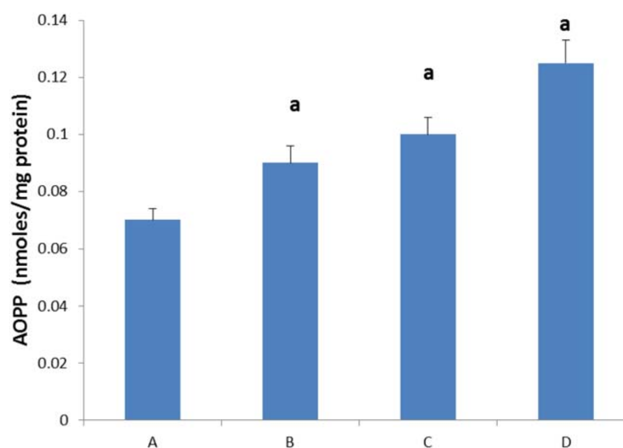

Fig. 4. Renal levels of advanced oxidation protein products (AOPP) following exposure to sodium fluoride. Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p < 0.05$, when, B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

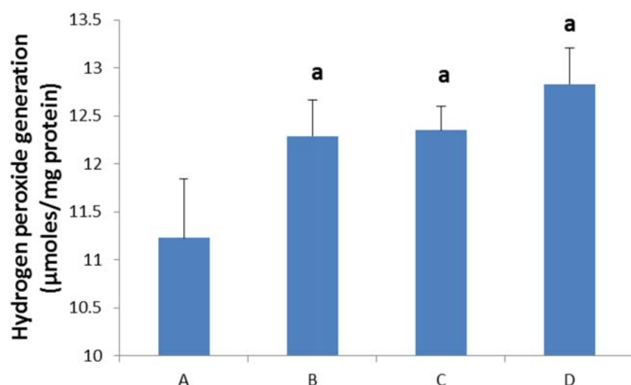

Fig. 5. Cardiac hydrogen peroxide (H2O2) generated following exposure to sodium fluoride. Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p < 0.05$, when, B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

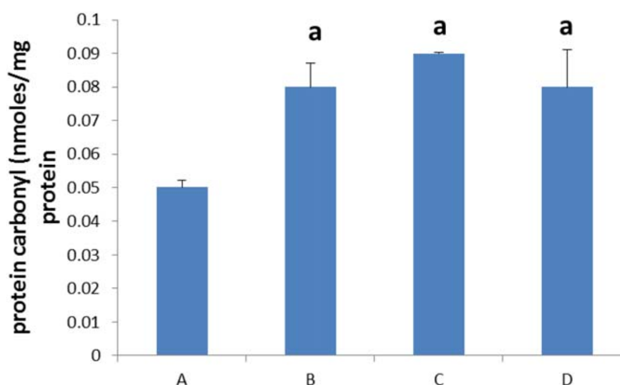

Fig. 7. Cardiac level of protein carbonyl following exposure to sodium fluoride (NaF). Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p<0.05$, when B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

oxidative stress and toxicity associated with NaF. The results from this study clearly demonstrated significant depletion of GSH and elevation of H_2O_2 which could be cytotoxic to renal and cardiac tissues. Increasing the dose of NaF also significantly increased the levels of AOPPs and protein carbonyl in the renal and cardiac tissues in exposed rats. Several studies have suggested elevated level of AOPPs in the tissues as a prognostic factor for severe cardiovascular disease and progression of renal damage (Cumaoglu et al., 2007; Negre-Salvayre et al., 2008; Iacobini et al., 2009). It is important to note that protein carbonyl assays generally measure any carbonyl in a protein, regardless of the source. Therefore, elevated levels of protein carbonyl are believed to be caused by increased oxidation of protein due to oxidative stress (Yaidikar and Thakur, 2015; Zhou et al., 2015). A significant increase in total thiol observed in the Kidney, whereas a significant decrease was observed in the non-protein thiol. This is indicative of enhanced protein oxidation in the renal tissue which might be associated with systemic oxidative stress (Manna and Jain, 2015; Wu et al., 2016). Therefore, the decrease observed in the non-protein thiol in the kidney may be associated with the observed renal toxicity.

The GPx and GST are key enzymes that take part in maintaining glutathione homeostasis in the tissues. GPx and GST as antioxidant enzymes work together with GSH in the decomposition of H_2O_2 and other organic hydroperoxides (Zhou et al., 2016). We therefore proposed that the participation of this array of antioxidant enzymes constituted a major

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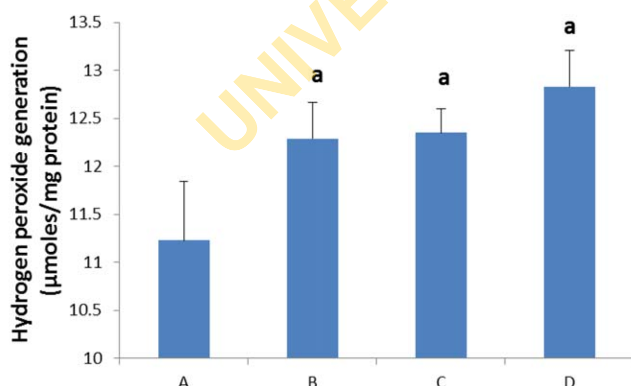

Fig. 6. Renal hydrogen peroxide (H2O2) generated following exposure to sodium fluoride (NaF). Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF), and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p < 0.05$, when, B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

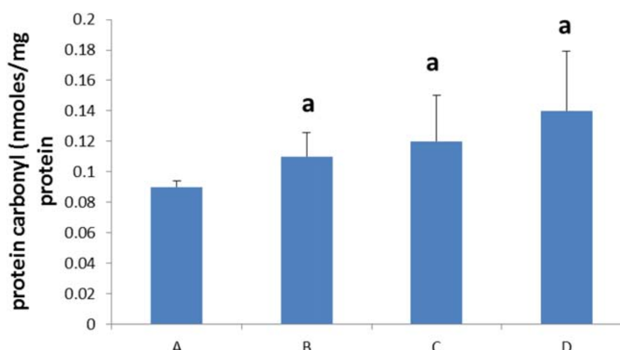

Fig. 8. Level of protein carbonyl in the renal tissue following exposure to sodium fluoride (NaF). Group A (Control), Group B (150 mg/L of NaF)), Group C (300 mg/L of NaF) and Group D (600 mg/L of NaF). Superscript (a) indicates significant difference at $p < 0.05$, when B, C, and D are compared with A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

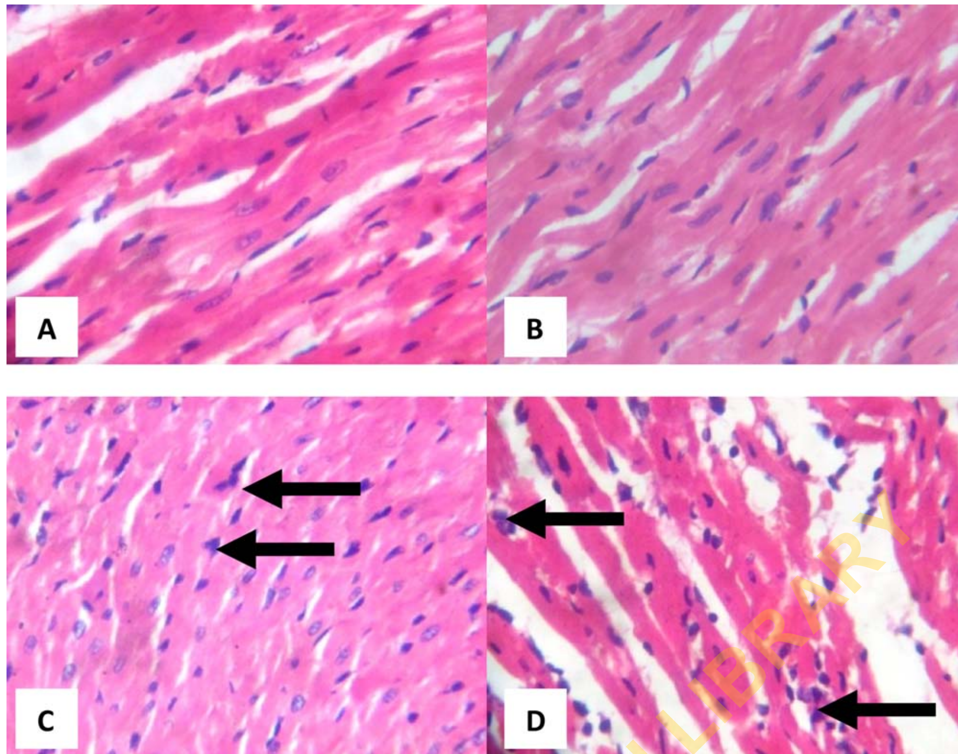

Fig. 9. The effect of sodium intoxication of cardiac tissue. Group A (Control) shows no visible lesion. Group B (150 mg/L of NaF) shows no significant lesion. Group C (300 mg/L of NaF) little infiltration of inflammatory cells whereas the rats in Group D (600 mg/L of NaF), show mild infiltration of the myocardial interstistium by inflammatory cells (black arrows). Histologic slides were stained with Hematoxylin & Eosin (magnification X100). Abbreviation: NaF (Sodium fluoride). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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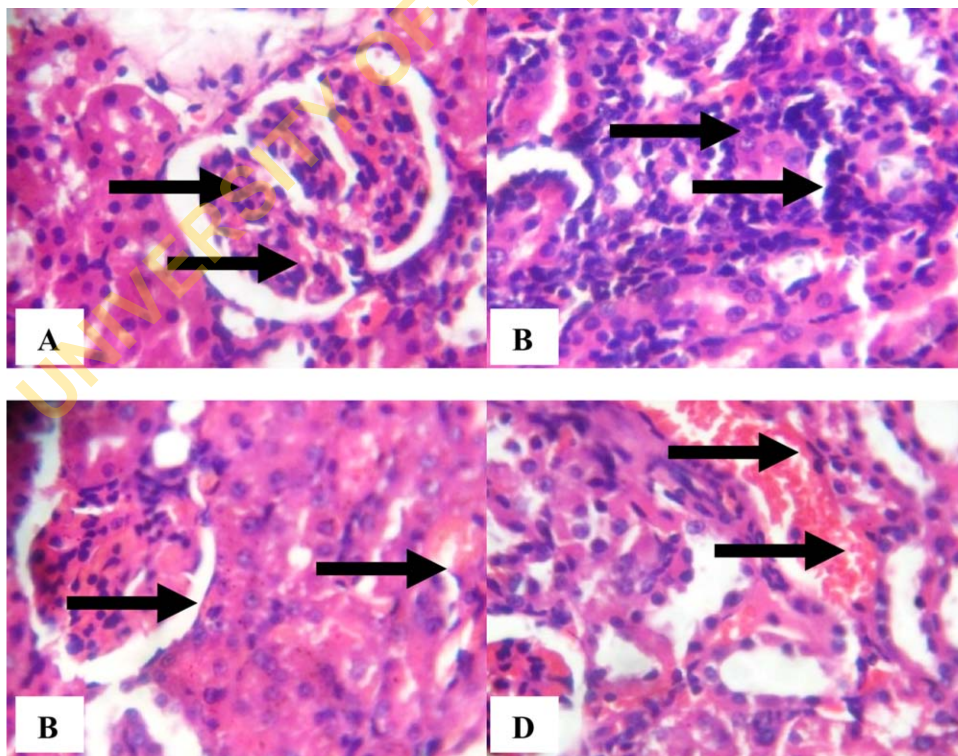

Fig. 10. The effect of sodium intoxication of renal tissue. Group A (Control) Shows normal architecture of the kidney with little inflammatory cells. Group B (150 mg/L of NaF) shows focal peritubular and periglomerular infiltration by inflammatory cells (black arrow) and fusion of the glomeruli with the capsule (black arrow). Group C (300 mg/L of NaF) show disjointed endothelial lining (black arrows) whereas the rats in Group D (600 mg/L of NaF) shows necrosis and congestion of vessels (black arrows). Histologic slides were stained with Hematoxylin & Eosin (magnification ×100). Abbreviation: NaF (Sodium fluoride). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

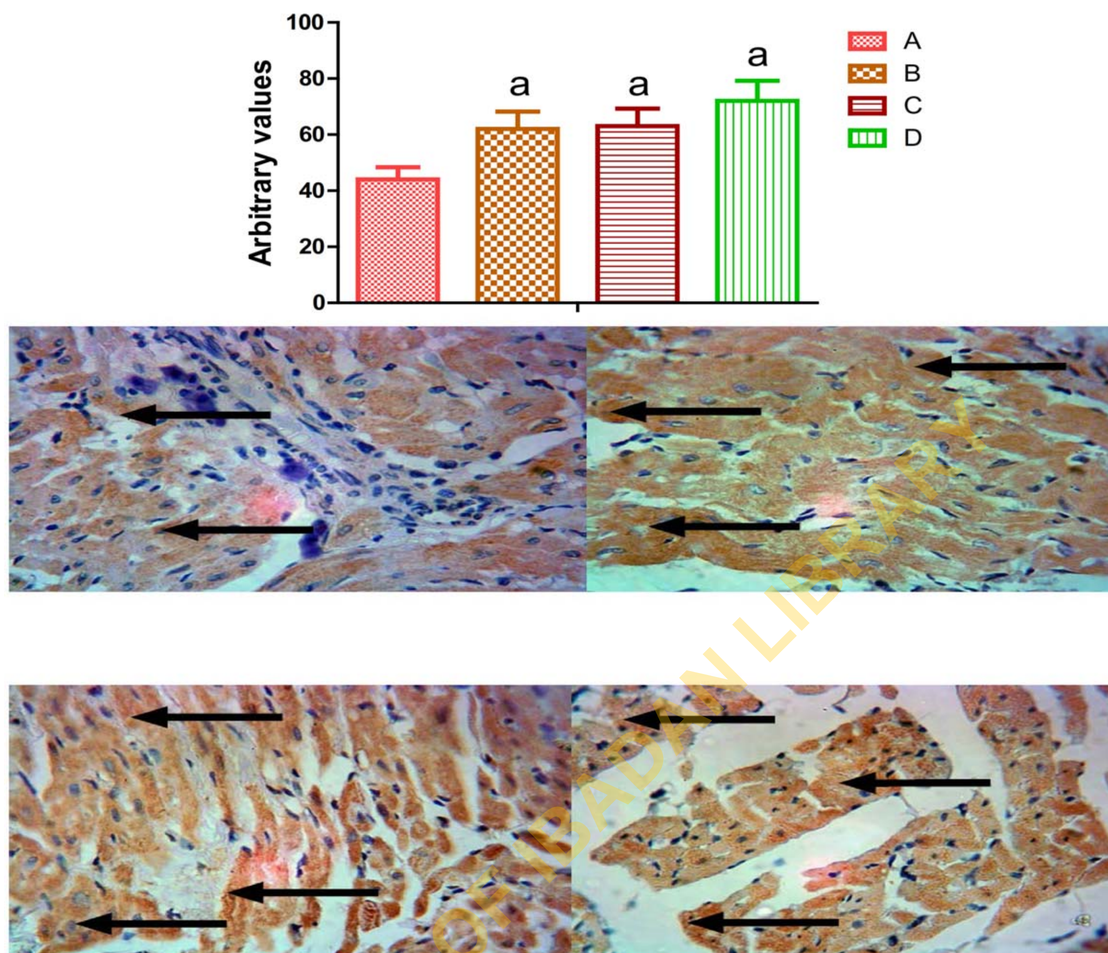

Fig. 11. The effect of sodium intoxication of cardiac NF-kB expressions. Group A (control) shows lower expressions of nuclear factor kappa beta (NF-kB) in the cardiac tissues. Group B (150 mg/L of NaF) shows higher expressions of NF-kB. Group C (300 mg/L of NaF) higher expressions of NF-kB than Groups A and B cells whereas the rats in Group D (600 mg/L of NaF), show expressions of NF-kB similar to that of Group C (black arrows). The slides were counterstained with high definition hematoxylin and viewed x 400 objectives (magnification X100). Abbreviations: NaF (Sodium fluoride), ppm (part per milion) or mg/L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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part of defense system against free radical mediated tissue or cellular damage after NaF exposure. According to the results of this study, significant changes were observed in the activities of endogenous antioxidant enzymes in the cardiac and renal tissues following exposure to NaF. There was reduction in the GPx activity in the heart, but only the group that received highest (600 ppm) dose of NaF showed significant decrease in GPx activity. At the end of exposure period, there was significant increase and decrease in the activity of GST in the heart and kidney of rats exposed to 150 ppm NaF, respectively. There was significant decrease in activities of SOD and catalase in the cardiac tissues while significant increase was observed in the renal tissues. The decrease in SOD and CAT may suggest that cardiac tissue has less protective/adaptive mechanism to NaF-induced oxidative damage while increase in the renal tissue indicated protective adaption via unknown mechanism.

Serum markers of cardiac damage also showed significant changes. The LDH, CK-MB, and AST activities in the serum increased significantly following exposure to NaF. Recent studies have revealed the importance of these enzymes as reliable biomarkers and prognostic factors for cardiac tissue damage (Geng et al., 2015; Liu et al., 2015; Sahu et al., 2016). BUN only showed a significant increase in the group that was exposed to 600 ppm NaF whereas dose-dependent significant increase in serum creatinine level was observed following exposure to sodium fluoride. This increase may be due to increase protein catabolism resulting from sodium fluoride-induced systemic oxidative damage. This suggests extensive glomerular damage and tubular epithelial cells damage which may also reduce the rate of creatinine clearance from the kidneys and equally its retention in the blood circulation (Seelhammer et al., 2016). The activities of serum

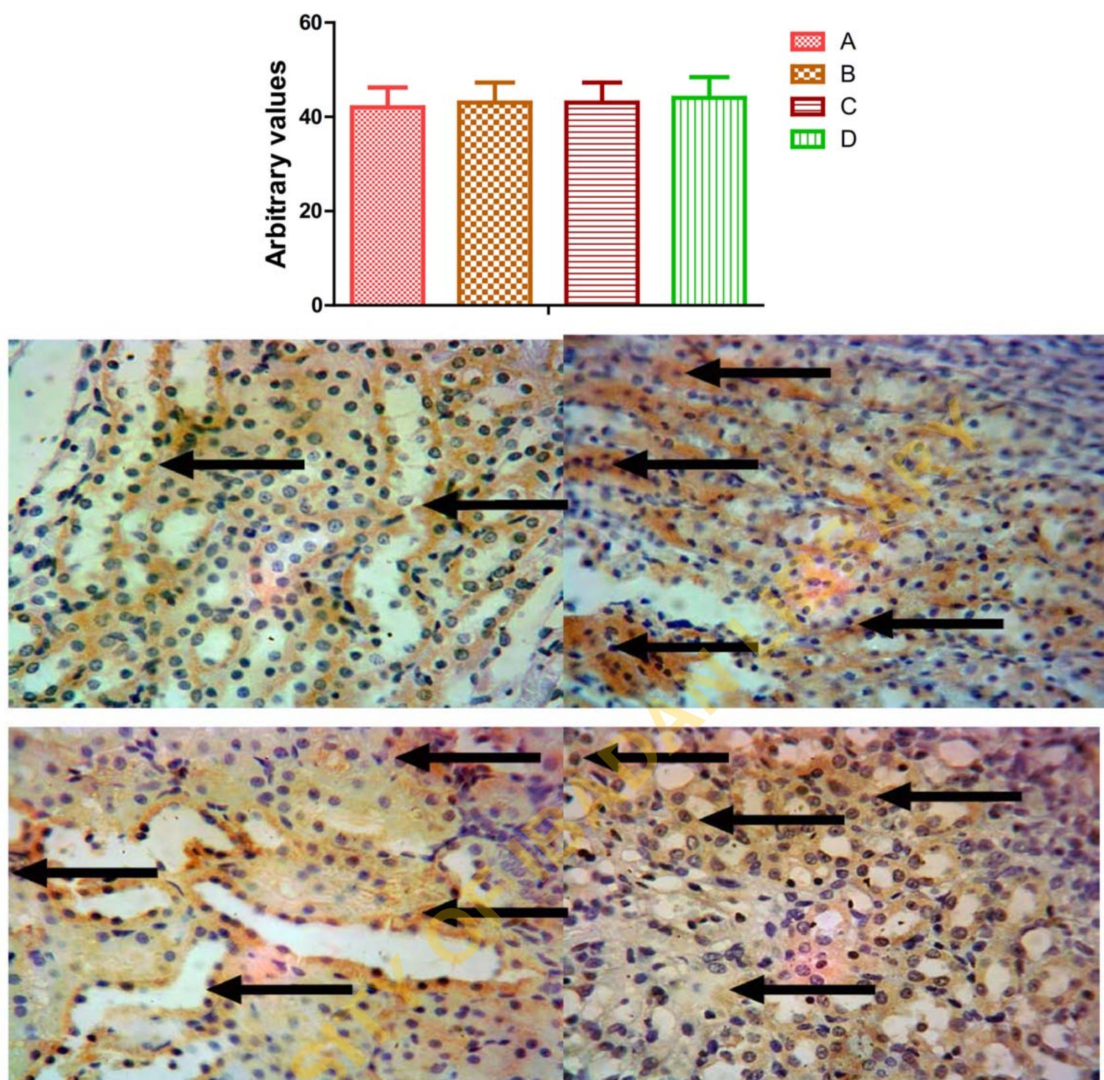

Fig. 12. The effect of sodium intoxication of renal NF-kB expressions Group A (control) shows lower expressions of nuclear factor kappa beta (NF-kB) in the renal tissues. Group B (150 ppm of NaF) shows higher expressions of NF-kB. Group C (300 ppm of NaF) higher expressions of NF-kB than Groups A and B cells whereas the rats in Group D (600 ppm of NaF), show expressions of NF-kB similar to that of Group C (black arrows). The slides were counterstained with high definition hematoxylin and viewed x 400 objectives (magnification $\times$100). NaF (Sodium fluoride), ppm (part per milion) or mg/L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

xanthine oxidase (XO) and myeloperoxidase (MPO) increased significantly following exposure to NaF. The increased activity of xanthine oxidase has been implicated in oxidative stress through generation of reactive oxygen species (Biberoglu et al., 2016). Similarly, the increased activity of XO may also be an indirect measurement of serum uric acid level. Recently, association between hyperuricaemia and renal failure has also been reported (Meng et al., 2015; Ohashi et al., 2015). In addition, the observable reduction in both renal and cardiac NO also contributed significantly to the development of hypertension. The reduced bioavailability of NO could be ascribed to the reduction in the activity of nitric oxide synthase or as a result of depletion of NO by superoxide anion radical. The reduced NO bioavailability has been documented to precipitate hypertension (Chalupsky et al., 2015). Furthermore, the MPO plays an essential role in inflammation and oxidative stress at the cellular level through its enzymatic action that leads to the generation of reactive molecules capable of oxidizing antioxidants, lipids and protein of LDL (Zang et al., 2015). The MPO has recently being recognized as a marker of heart failure and cardiac damage apart from its involvement in inflammation and oxidative stress (Kennedy et al., 2015). Hence, elevated serum XO and MPO activity could be suggestive of renal and cardiac damage.

Histologically, cardiac tissue of NaF exposed rats showed mild pathology, as indicated by mild infiltration of the myocardial interstistium by inflammatory cells. In contrast, the kidney of rats exposed to NaF showed significant lesions including focal peritubular and periglomerular infiltration by inflammatory cells, fusion of the glomeruli with the capsule. Focal area of haemorrhagic lesion and congestion of vessels were observed in the group that was exposed to 300 ppm NaF, whereas the rats in the group that was exposed to 600 ppm NaF showed necrosis and congestion of vessels. The pathological findings observed at histology further confirm the nephrotoxicity and cardiotoxicity of NaF.

The immunohistochemistry of renal and cardiac NF-kB expressions revealed higher expressions NF-kB in rats exposed to NaF. The activation of NF-κB by oxidative stress has been associated with ventricular remodeling and arterial hypertension (Li et al., 2015; Udwan et al., 2016). Similarly, increased activity of NF-κB has been reported as an important factor in the pulmonary vasoconstriction and structural remodeling (Yang et al., 2015). The expressions of NF-kB in this study were higher in rats administered NaF compared to the control. This was suggestive of the involvement of NF-kB signaling in cardiorenal dysfunction following NaF toxicity. Oxidative stress is one of the activators of NF-κB, resulting in the release of NF-κB from cytosolic inhibitors and its translocation to the nucleus and subsequently to the expression of NF-κB-target genes (Park, 2015). This over expression of NF-kB in these tissues clearly indicates inflammation, cardiac and renal damage mediated by oxidative stress due to NaF intoxication. In line with the observations in this study, caution should be exercised in the use of NaF in dental hygiene to prevent potential health hazards including renal failure and cardiovascular complications associated with NaF.

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