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Modulatory effects of gallic acid on sodium fluoride induced nephrotoxicity in the wistar rats

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Abstract
Background: Exposure to environmental chemical contaminants such as Sodium Fluoride appears to be a regular part of life. It is therefore, necessary to limit their effects through the use of natural products.
Objective: To evaluate the modulatory/protective effect of gallic acid on the nephrotoxic effect of sodium fluoride in rats.
Materials & Methods: The study included four groups of twenty-four adult male Wistar, weighing 220 ± 5.6 g. The rats were divided into four groups, A – D consisting of six rats per group. Group A, was given distill water, while group B, received 300 ppm of NaF only, Group C, 300 ppm NaF and 60 mg/kg of gallic acid, group D, 300 ppm NaF and 120 mg/kg of gallic acid per os for 7 days. Samples were collected for haematology; biochemistry and histology.
Results: The PCV and RBC values were higher in the rats exposed to NaF and 120 mg/kg of gallic acid while the MCV was lower in NaF alone. Significant signs of neutropenia and kidney damage and inflammation were also observed which were ameliorated by gallic acid.
Conclusion: The study further established that NaF induces kidney damage by increasing inflammation in the soft tissue, which was attenuated by gallic acid.

Keywords: sodium fluoride, inflammation, gallic acid, Wistar rats

Introduction
Sodium fluoride (NaF), despite its widely reported nephrotoxicity is still a widely used compound in household materials, including toothpaste; and in fluoridation of potable water for human and animal consumption in the prevention of dental caries (Pain GN 2017) [1]. Human exposure to fluoride also occurs through consumption of food materials contaminated by industrial wastes and its continual usage in municipal drinking water, in developed (Kohn WG *et al*., 2001, Allukian M *et al*., 2017) [2, 3] and developing countries alike (Whyte MP *et al*., 2008, Waugh DT *et al*., 2016) [4, 5]. For example, fluoridation of drinking water is mandatory, and backed by legislation in the Republic of Ireland, which, coupled with elevated fluoride consumption in tea has considerably increased exposure to fluoride in this country (Waugh DT *et al*., 2016) [5]. Fluorosis, a clinical condition associated with fluoride poisoning has also been widely reported as an epidemic condition in India, has resulted in several measures by the government to deal with the impasse (Quadri JA *et al*., 2016) [6].

Sodium fluoride-induced nephrotoxicity has been associated with increased the generation of reactive oxygen species and other free radical levels and depletion of antioxidant defense system in the body (Hamza-Reham Z *et al*., 2015) [7]. Several authors have reported decreased glutathione peroxidase, glutathione reductase, glutathione S-transferase, superoxide dismutase and catalase in liver, kidney and brain tissues of experimental rats exposed to NaF (Hamza Reham *et al*., 2015, Goschorska M *et al*., 2017, Bharti V *et al*., 2017) [7, 8, 9].

As postulated by Song C *et al*. (2017) [10], Sodium fluoride-induced nephrotoxicity and oxidative damage is believed to occur by the damaging effect of NaF on Peroxisome proliferator-activated receptor-coactivator 1α (PGC-1α) and its inhibition of nuclear factor erythroid 2 (NF-E2)-related factor 2 (NRF2) – a transcriptional co-activator. Down regulation of NRF 2 subsequently results in the reduction Sirtuin 3 (Sirt3) transcription. Sirtuin 3 is the primary mitochondrial acetyl-lysine deacetylase that modulates various proteins to control mitochondrial reactive oxygen species (ROS) levels (Pi H *et al*., 2015) [11]. A reduction in Sirt3 decreases the deacetylation of the transcription factor – Forkhead box O3a (FoxO3a), and superoxide dismutase 2 (SOD2), resulting in ROS accumulation and cellular apoptosis in the renal tubules (Song C *et al*., 2017) [10]. Fluoride also induces oxidative damage in the CNS as it readily crosses the blood brain barrier, causing neurodegenerative diseases by ROS accumulation and excitotoxicity – a persistent overstimulation and damage of neurons (Goschorska M *et al*., 2017) [8].

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Gallic acid (3,4,5-trihydroxybenzoic acid), is a potent phenolic antioxidant generally found in nuts, green tea, hops, grapes, red wine and oak bark. It can also be obtained from the hydrolysis of tannins, although this is quite expensive (Safaei F *et al.*, 2017) [12]. Gallic acid isolated from *Peltiphyllum peltatum* has been previously used for amelioration of NaF induced oxidative damage in the brain (Nabavi SF *et al.*, 2012) [13] either as a form of pretreatment, concurrent or post exposure to NaF (Nabavi SM *et al.*, 2013) [14]. Other recent studies have also highlighted the anti-inflammatory (Kim SH *et al.*, 2005, Karimi-Khouzani O *et al.*, 2017, BenSaad LA *et al.*, 2017, Moschona A *et al.*, 2017) [15-18], anticancer effects (Weng YP *et al.*, 2017, Lee HL *et al.*, 2017) [19, 20] as well as protective activities of gallic acid in cancer therapy (Safaei F *et al.*, 2017, Omobowale *et al.*, 2017) [12, 21].

The use of antioxidants such as gallic acid in the amelioration of NaF toxicity has become necessary and may find its way into regular human supplement because of the ubiquitous nature of fluoride in our environment and in drinking water. The present study was therefore designed to evaluate the haematotoxicity, hepatotoxicity and nephrotoxicity effect of NaF and the protective effects of gallic acid on NaF induced toxicity in the rats in the hot humid tropical environment.

Materials and Methods
Reagents and chemicals
All chemicals and reagents used are of analytical grade. Commercial reagent kits for the assay of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferases (ALT), Creatinine phosphokinase (CPK), total cholesterol (TC), total protein (TP), triglyceride (TAG), and high-density lipoprotein (HDL), Low density lipoprotein (LDL), Urea, Creatinine were as supplied by Randox Diagnostics, Crumlin, United Kingdom

Animal Model
Twenty adult male Wistar rats weighing an average of 135 ± 5.5 gm were used for the study. The animals were procured and kept from the experimental animal unit of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine to acclimatize for a period of two weeks. During this period, the animals were fed with standard rat chow while potable water was provided, *ad libitum.* At the commencement of the experiment, the rats were weighed and divided into four groups A – D, consisting of five animals per group. Group A, which served as the control received

Experimental Design
Animals in Group A, which served as the positive control were given only distilled water while those in Groups B – D were given 300 ppm of sodium fluoride (NaF) by gastric gavage. Furthermore, those animals in Group C and D were given 60 and 120mg/kg gallic acid, also by gastric gavage. All treatment lasted for a period of 7 days, after which the experiment was terminated.
The study followed all international and national standard procedure as required for the maintenance of animal welfare. The study was also approved by the University of Ibadan Animal Care, Use and Ethics Committee of the Faculty of Veterinary Medicine.

Sample collection
At the termination of the experiment, blood samples were collected from each rat through the retro-orbital venous

plexus into heparinized bottles for haematology and plasma biochemistry. Three animals were thereafter randomly selected in each group and sacrificed by cervical dislocation for collection of samples for histopathological examination.

Haematology
Haematological parameters – packed cell volume (PCV), haemoglobin concentrations (Hb), red blood cell counts (RBC), white blood cell counts (WBC) and platelet counts as well as differential leucocytes counts were determined by Sysmex Kx-21 (Canada) automatic haematology analyzer. Erythrocyte indices – mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were determined from the PCV, Hb and RBC values.

Plasma Biochemistry
The levels of plasma total protein (TP) were determined by the Biuret method as described by Janairo G *et al.* (2015) [22]. Lipid profile including total cholesterol (TC), triglyceride (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined using Randox assay kits, Randox Diagnostics, Crumlin, United Kingdom. Plasma electrolytes – sodium, potassium, chloride, and bicarbonate were determined by potentiometry method (Megahed AA *et al.*, 2016) [23].

Histopathology
Heart and kidney sections collected from representative of each test groups and the control were fixed in 10% neutral buffered formalin (10% formalin in 0.08 M Sodium phosphate at pH 7.4). Samples were prepared for histology using haematoxylin and eosin (H and E) stain after dehydration in graded alcohol and embedded in paraffin at 60 °C inside labeled paraffin embedding molds. The molds were then sectioned using a microtome at 5 μm thickness and each section floated in 45 °C water bath to allow crinkled part to spread before they are placed on glass slides. The slides were then stained in haematoxylin and eosin using standard protocol. Slides were examined using Olympus light microscope.

Statistical Analysis
All data are presented as mean ± SD. Mean values were compared by One-way ANOVA, with Tukey's post hoc test for multiple comparisons between groups, using GraphPad Prism statistical software, version 5.01 for Windows, GraphPad Software, San Diego California, USA (https://www.graphpad.com). A probability value of 0.05 and below was considered to be significant.

Results
Haematology
The effects of exposure to NaF and gallic acid on the erythrocytic and leucocytes parameters of the Wistar rats are shown in Tables 1 and 2, respectively. The PCV in the rats that were exposed to NaF only (Group B) and those exposed to NaF plus 60 mg/kg gallic acid (Group C) were marginally higher than the control while those in Group D with NaF 120 mg/kg gallic acid was significantly higher ($P < 0.05$) than the untreated control (Table 1). The RBC value of 6.83 ± 0.39 x $10^6/\mu L$ in the untreated control (Group A) was also significantly lower than the value of 8.44 ± 0.32 x $10^6/\mu L$ obtained in Group C ($P < 0.05$). It was also significantly lower ($P < 0.01$) than 8.44 ± 0.67 x $10^6/\mu L$ obtained in Group

D. The mean RBC value in Group B that received NaF only was also higher than the untreated control, although, non significantly. The mean corpuscular volume (MCV) was however higher significantly in the untreated control than in Groups C and D (P < 0.05).

Table 1: The effects of NaF alone and in combination with gallic acid on the Erythrocytic indices of Wistar rats.

Parameters	Group A	Group B	Group C	Group D
PCV (%)	$41.67\pm2.12^{a*}$	45.67 ± 2.08	46.33 ± 0.58	47.00 ± 2.00^{a}
RBC (x10^6/µL)	$6.83\pm0.39^{a*b**}$	7.83 ± 0.31	8.25 ± 0.32^{a}	8.44 ± 0.67^{b}
Hb (g/dl)	14.34 ± 1.36	15.43 ± 0.57	16.00 ± 0.26	16.10 ± 0.7
MCV (fl)	$60.72\pm0.53^{a*b*}$	58.31 ± 1.17	56.20 ± 1.50^{a}	55.82 ± 2.33^{b}
MCH (ρg)	20.71 ± 1.09	19.70 ± 0.06	19.42 ± 0.67	19.12 ± 0.79
MCHC (g/dl)	33.96 ± 1.59	33.81 ± 0.78	34.53 ± 0.44	34.26 ± 0.03

Values are expressed as means ± SD.
Values with the same superscript alphab*et al*ong the same row are significantly different. * = P < 0.05, ** = P < 0.01 and *** = P < 0.001.

As shown in Table 2, only the differential neutrophil count showed significant difference across the groups of the rats exposed to NaF only and in combination with gallic acid. For example, the mean differential neutrophil count in those rats treated with NaF only (Group B) was significantly lower than the value obtained in the untreated control (P < 0.01), as well as Group C (P < 0.001) and Group D (P < 0.05).

Table 2: Effects of NaF exposure alone and in combination with gallic on platelet and Leucocyte parameters in Wistar rats.

	Group A	Group B	Group C	Group D
WBC (x 10^3/µL	12.87 ± 1.5	8.90 ± 0.36	13.51 ± 1.91	11.41 ± 1.46
Neut (x 10^3/µL)	$5.51\pm0.26^{a**}$	2.77 ± 0.38^{abc}	$4.98\pm0.68^{b**}$	$4.25\pm0.51^{c*}$
Lymph (x 10^3/µL)	7.33 ± 1.79	6.07 ± 0.24	8.43 ± 1.43	7.12 ± 2.00
Mono (x 10^3/µL)	0.004 ± 0.006	0.006 ± 0.01	0.005 ± 0.008	0
Eos (x 10^3/µL)	0	0	0.005 ± 0.009	0.004 ± 0.006
Baso (x 10^3/µL)	0	0	0	0
Plat (x 10^3/µL)	706.67 ± 135.77	588.67 ± 90.01	641.67 ± 86.94	681.00 ± 149.81

Values are expressed as means ± SD.
Values with the same superscript alphab*et al*ong the same row are significantly different. * = P < 0.05, ** = P < 0.01 and *** = P < 0.001.

Plasma biochemistry
Total plasma urea was also higher in those rats that were treated with NaF only than the values obtained in the untreated control at P < 0.05, but the other metabolite evaluated (creatinine) and electrolytes – sodium, chloride, potassium and bicarbonate ions had similar values across the groups (Table 3).
Table 4 shows the mean plasma protein and lipoprotein values as influence by treatment with NaF alone, and in combination with gallic acid. The total cholesterol values increased significantly in the treated groups. For instance, total plasma cholesterol value in the untreated control was significantly lower (P < 0.05) than that of the rats treated with NaF only. It was also significantly lower (P < 0.001) than the values obtained in Group C (NaF+ 60 mg/kg gallic acid) and Group D (NaF+120 mg/kg gallic acid) at P < 0.001. The total cholesterol in Group B (NaF only) was also lower than that obtained in Group D (P < 0.01). Following closely after the trend observed in the total cholesterol, the mean triglyceride value was significantly lower (P < 0.05) than the values in Group C. It was also lower, though non-significantly than the values in Groups B and D. The high-density lipoprotein (HDL) and low-density lipoprotein (LDL) on the other hand showed significant variation across the treatment groups. For example, HDL value in the untreated control was significantly lower than Group C (P < 0.05) and Group D (P < 0.001). Meanwhile HDL value in Group B was also significantly lower than that of Group D (P < 0.05). In like manner, the LDL value in the untreated control was significantly lower than that of Group C that were treated with NaF + 60 mg/kg gallic acid (P < 0.05) and Group D that were treated with NaF + 120 mg/kg gallic acid (P < 0.001).
Fig 1 – 4 and Table 5 show the results of the plasma gonadotropins – LH and FSH, prolactin and testosterone in the rats following exposure to NaF and gallic acid. Plasma LH and FSH were observed to have fallen in the rats in Group B, relative to the control and those groups exposed to gallic acid. But the variation was observed were not statistically significant. The values of prolactin were however similar in all the groups.

Table 3: Plasma electrolyte and metabolite values of the Wistar rats exposed to NaF alone and in combination with gallic acid

	Group A	Group B	Group C	Group D
Na$^+$ (mmol/L)	139.00 ± 1.73	140.00 ± 2.00	140.00 ± 1.00	139.70 ± 2.52
K$^+$ (mmol/L)	3.87 ± 0.25	3.93 ± 0.21	3.90 ± 0.26	3.90 ± 0.26
Cl$^-$ (mmol/L)	106.70 ± 2.89	105.00 ± 5.00	106.70 ± 2.89	105.00 ± 5.00
HCO$_3^-$ (mmol/L)	21.67 ± 1.16	22.33 ± 2.08	22.00 ± 2.00	22.00 ± 1.00
Urea (mmol/L)	$24.67\pm3.51^{a*}$	31.00 ± 2.00^{a}	30.67 ± 2.08	29.33 ± 1.53
Creatinine (i.u/L)	0.60 ± 0.10	0.77 ± 0.06	0.73 ± 0.06	0.77 ± 0.06

Values are expressed as mean ± SD
Values with the same superscript alphab*et al*ong the same row are significantly different. * = P < 0.05, ** = P < 0.01 and *** = P < 0.001.

Table 4: Effects of NaF alone and in combination with gallic acid on the Plasma protein, enzymes and lipoprotein values adult ale Wistar rats.

	Group A	Group B	Group C	Group D
Total protein (g/dl)	7.03±0.11	7.30±0.10	7.20±0.20	7.17±0.15
Albumin (g/dl)	4.00±0.10	4.17±0.21	4.10±0.26	4.03±0.38
Globulin (g/dl)	3.03±0.06	3.13±0.15	3.10±0.10	3.13±0.23
Total bilirubin (umol/L)	0.57±0.11	0.60±0.20	0.53±0.25	0.47±0.15
Conj bilirubin (umol/L)	0.23±0.06	0.17±0.11	0.23±0.11	0.27±0.11
Total cholesterol (mmol/L)	93.67±12.66[abc]	115.00±5.00[a*d**]	135.30±5.03[b***]	144.30±5.86[c***d]
Triglyceride (mmol/L)	46.33±4.16[a*]	61.00±10.54	67.00±9.00[a]	66.00±6.24
HDL (mmol/L)	31.00±2.00[a*]	33.00±7.00[b*]	35.67±4.73	46.00±4.36[ab]
LDL (mmol/L)	68.67±4.16[a*b***]	73.67±6.43[c**]	95.33±13.32[a]	115.00±8.72[bc]

Values are expressed as mean ± SD
Values with the same superscript alphab*et al*ong the same row are significantly different. * = P < 0.05,
** = P < 0.01 and *** = P < 0.001.

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; AST, aspartate transaminase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase and ALP, alkaline phosphatase.
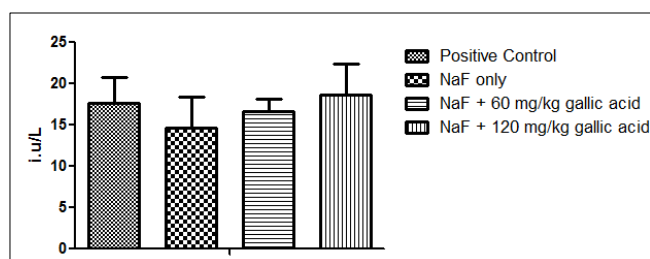

Fig 1: Plasma luteinizing hormone (LH) values in the Wistar rats exposed to NaF alone and in combination with gallic acid. Values are means while, vertical bars represent SD.
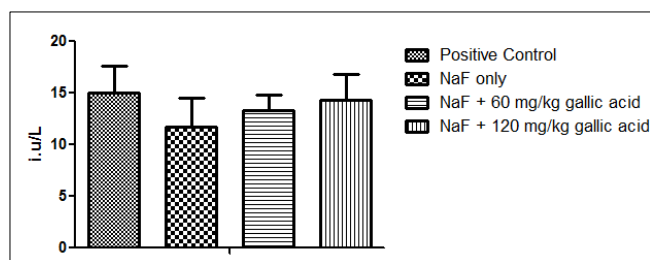

Fig 2: Plasma follicle stimulating hormone (FSH) values in the Wistar rats exposed to NaF alone and in combination with gallic acid. Values are means while, vertical bars represent SD.
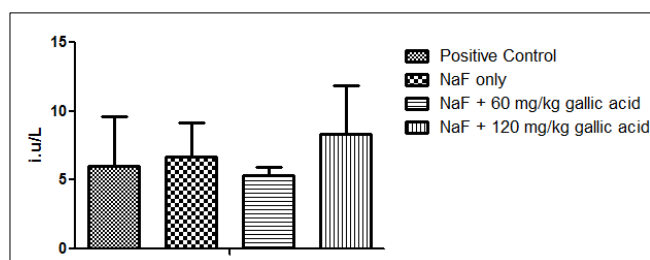

Fig 3: Plasma prolactin values in the Wistar rats exposed to NaF alone and in combination with gallic acid. Values are means while, vertical bars represent SD.
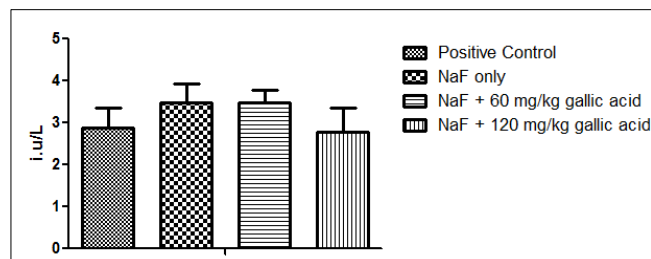

Fig 4: Plasma testosterone values in the Wistar rats exposed to NaF alone and in combination with gallic acid. Values are means while, vertical bars represent SD.

Table 5: Plasma Gonadotropins (luteinizing hormone (LH), follicle stimulating hormone (FSH)), prolactin (PRL) and testosterone values in the Wistar rats exposed to NaF alone and in combination with gallic acid

	Group A	Group B	Group C	Group D
LH (i.u/l)	17.67±3.05	14.67±3.78	16.67±1.53	18.67±3.78
FSH (i.u/l)	15.00±2.64	11.67±2.89	13.33±1.53	14.33±2.52
PRL (i.u/l)	6.00±3.60	6.67±2.52	5.33±0.58	8.33±3.51
Testosterone (i.u/l)	2.87±0.49	3.47±0.45	3.47±0.30	2.77±0.58

Histopathological findings
Histomorphology of the hearts and kidneys sampled from rats from each of the groups and the control is shown in Fig 5 – 12. Mild to moderate inflammatory cells infiltration of the myocardial tissues especially in Group B, which received NaF only. The inflammatory cells infiltration was however abated in the groups that were treated with gallic acid, especially at 120 mg/kg, in a manner that was similar top the untreated control. Significant periglobular and peritubular inflammatory cellular infiltration as well as eosinophilic substance infiltration were also observed in the NaF treated rats. This was slightly abated in the NaF plus gallic acid groups, especially at 60 mg/kg gallic acid, although the restoration was not entirely similar to the unexposed control.
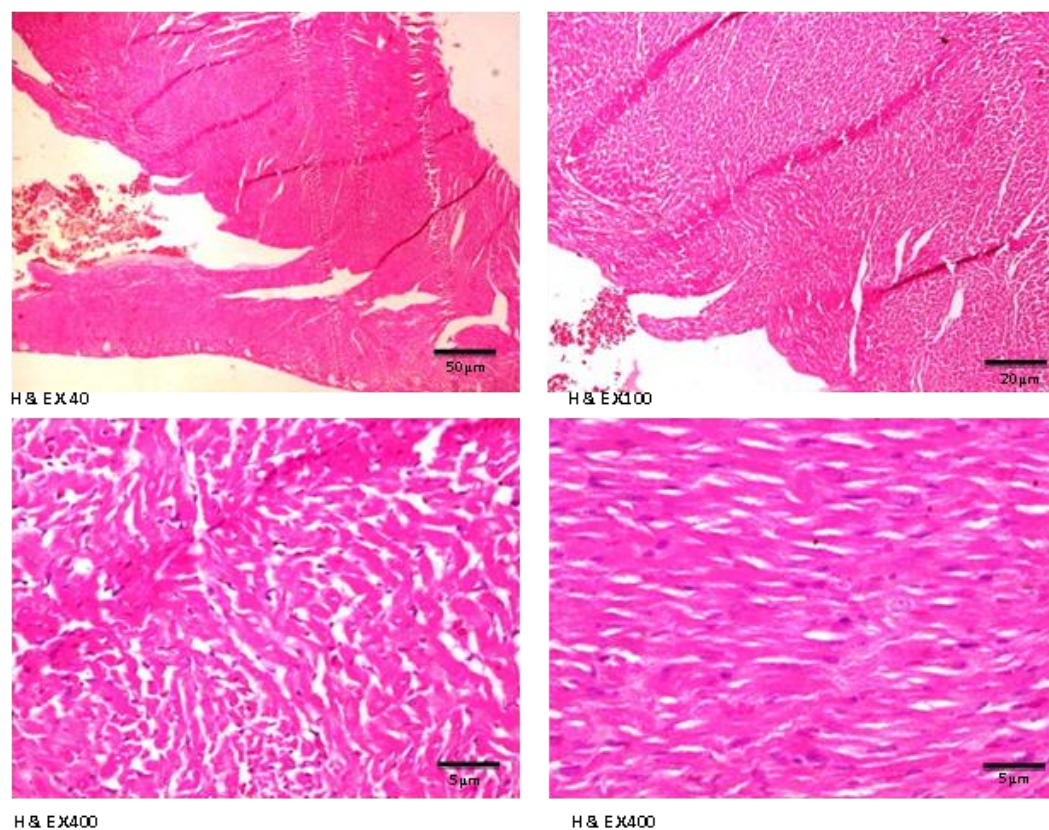
Fig 5: Photomicrograph of the heart in rats in normal untreated control, showing no visible lesions
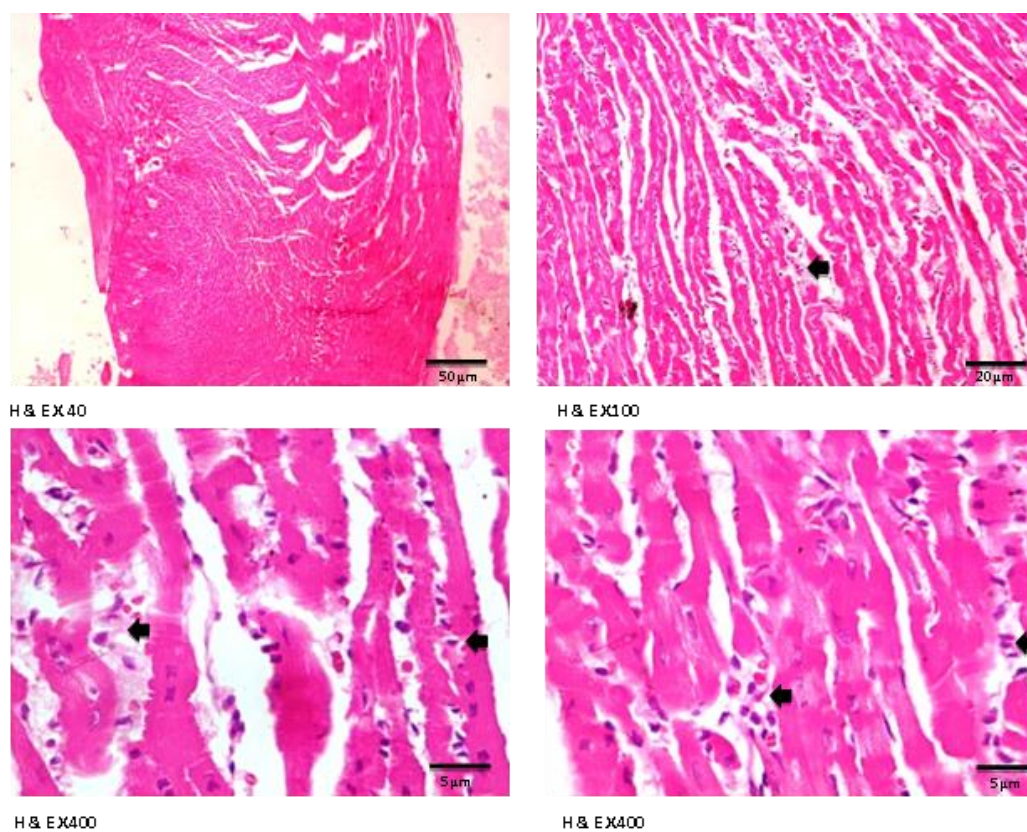

Fig 6: Photomicrographs of cardiac tissue of rat exposed to NaF only showing mild infiltration of inflammatory cells (black arrow) into the myocardium
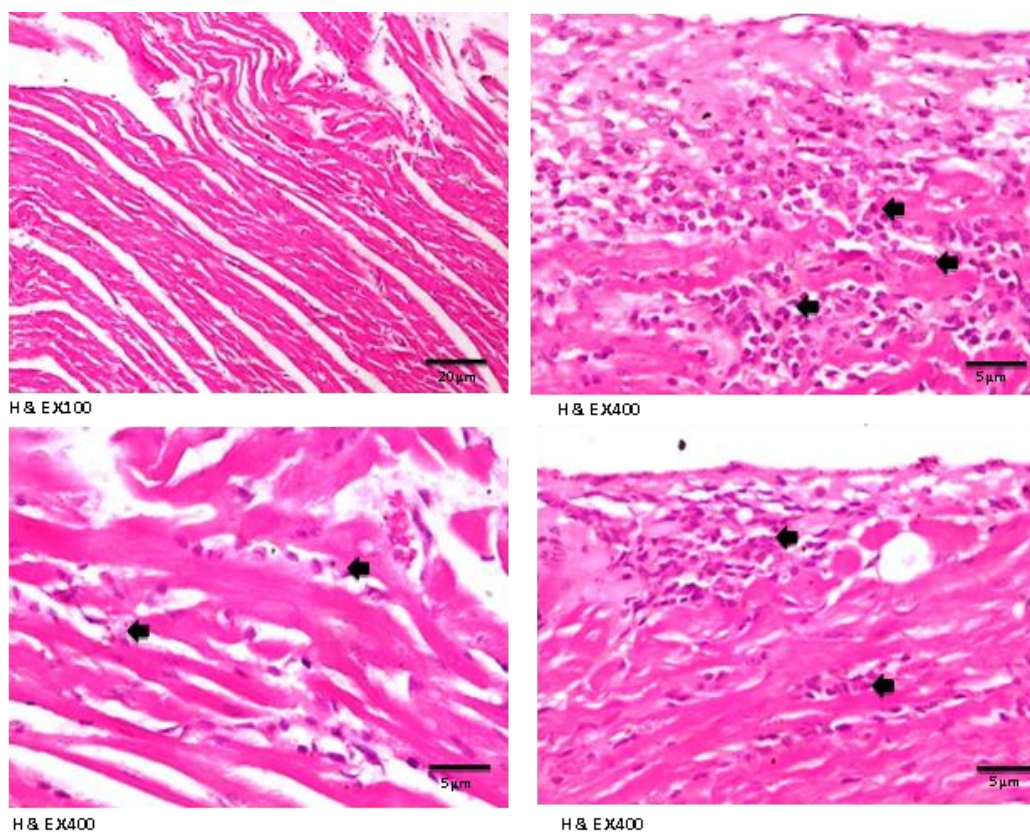
Fig 7: Photomicrographs of cardiac tissue of rats in Group C treated with NaF and 60 mg/kg gallic acid showing moderate infiltration of inflammatory cells to the endocardium (black arrows). There is mild inflammation of the myocardium
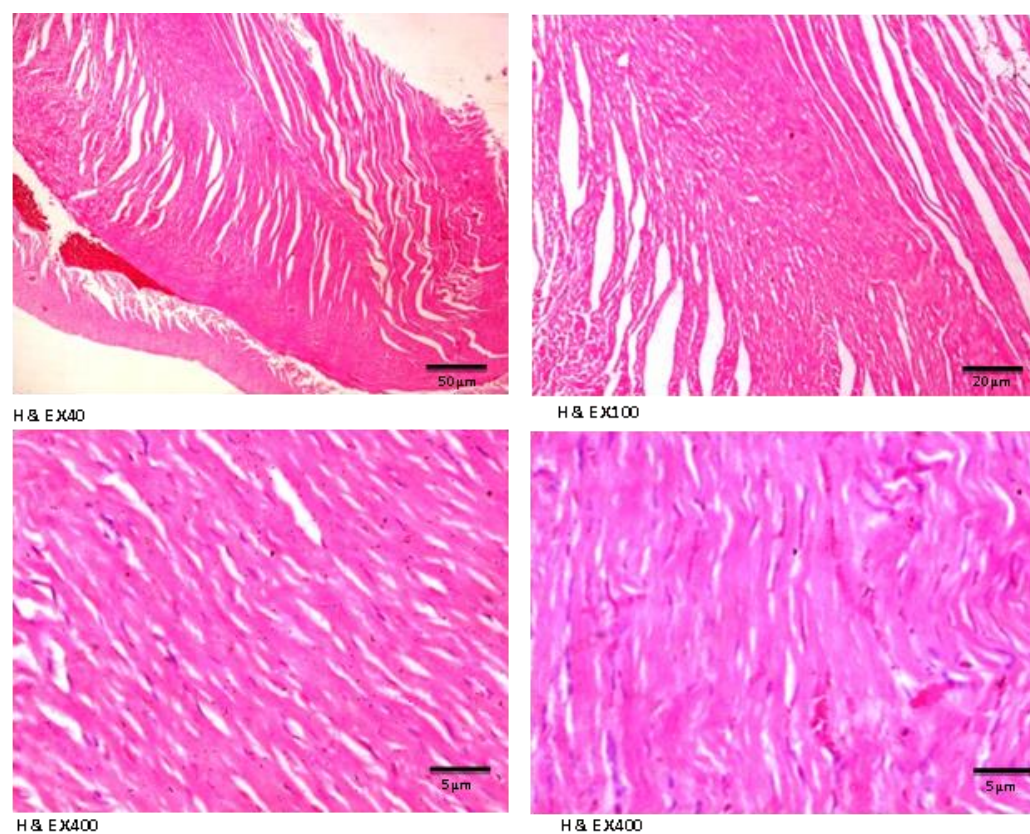

Fig 8: Photomicrographs of cardiac tissue of rats in Group D treated with NaF and 120 mg/kg gallic acid showing no visible lesion
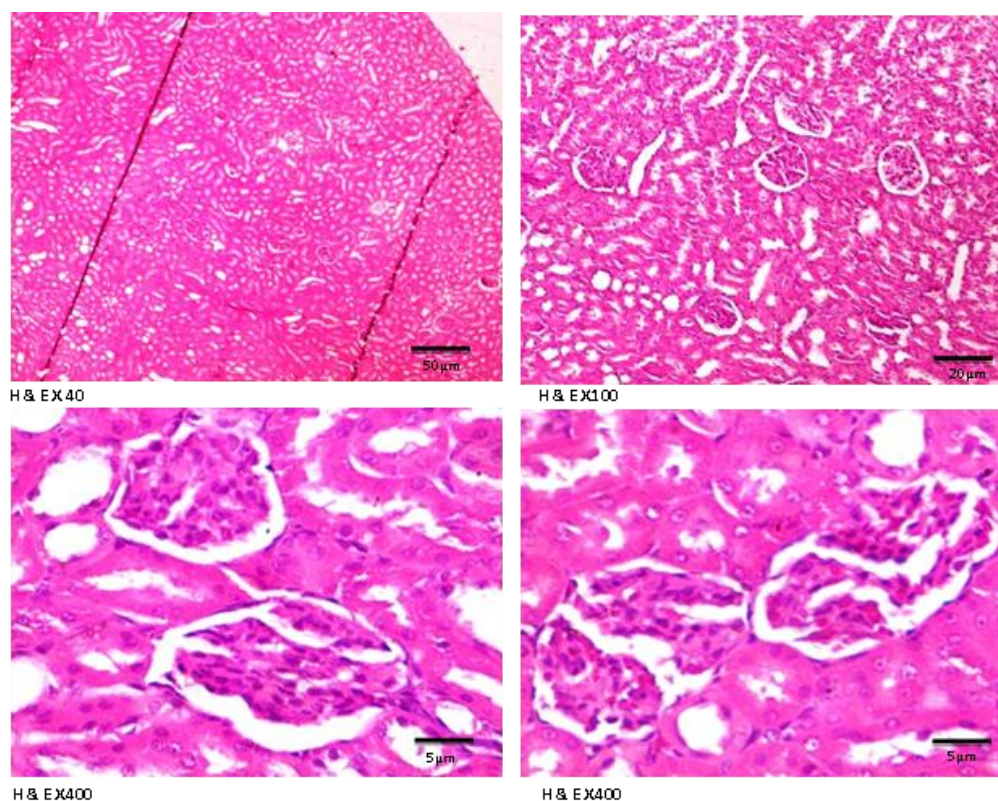
Fig 9: Photomicrograph of the kidneys of normal untreated control rats, showing normal bowman's capsules, glomeruli and tubules with no visible lesions
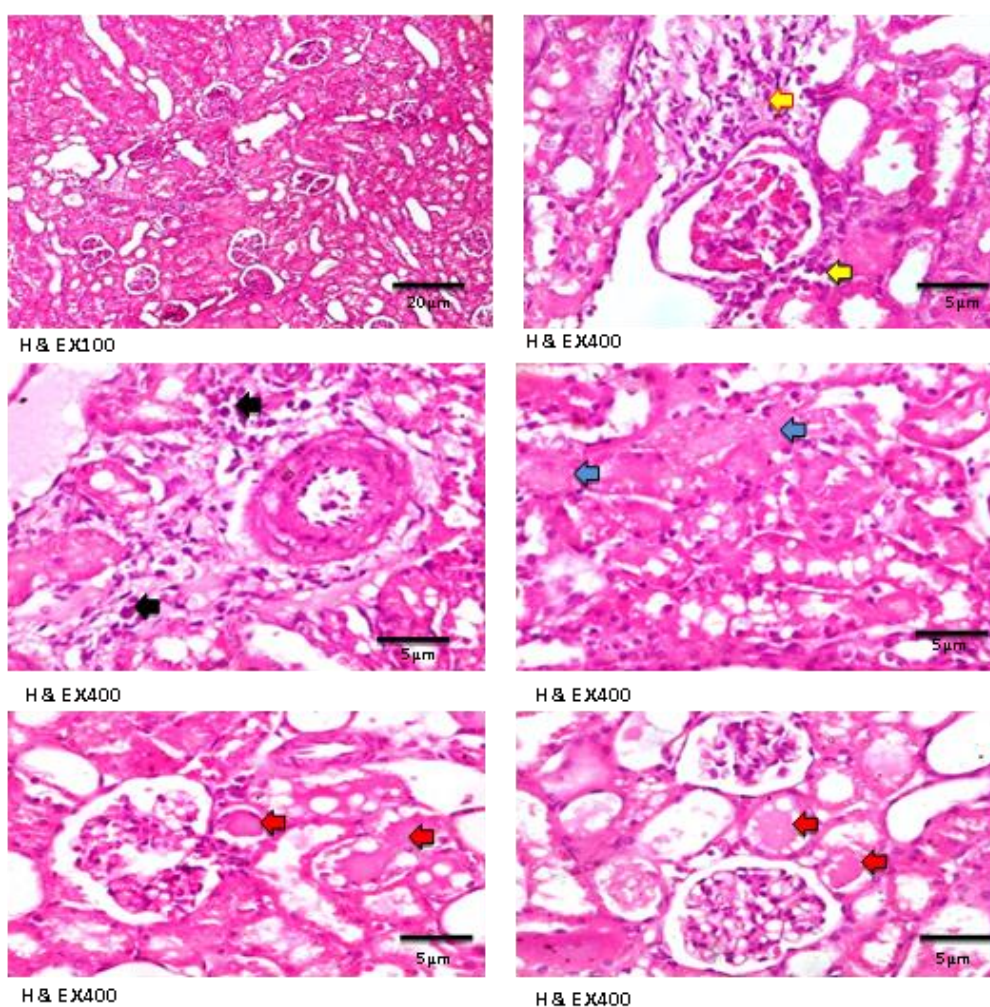

Fig 10: Photomicrographs of renal tissue of rats treated with NaF only, showing mild perivascular inflammation (black arrow) and mild periglomerular inflammation (yellow arrows). There were also focal area of attenuation of the tubules (blue arrow) and mild eosinophilic substance in the tubules (red arrows)
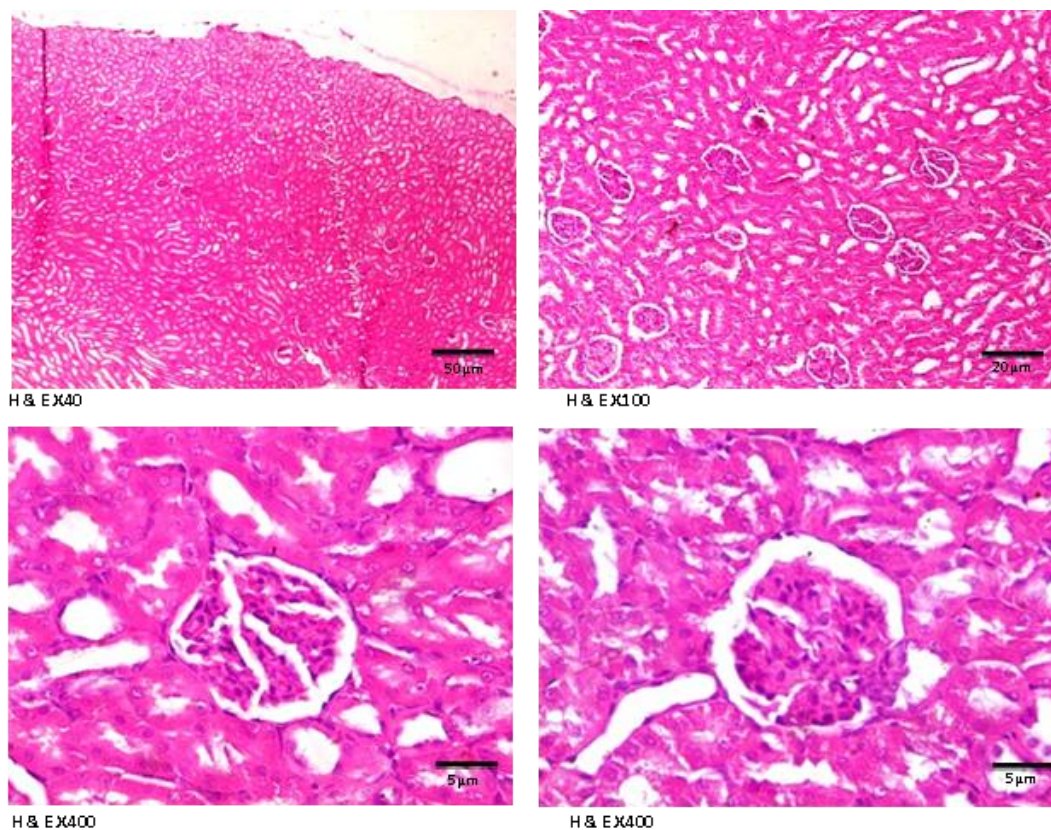
Fig 11: Photomicrographs of nephrotic tissue from rats treated with NaF and 60 mg/kg gallic acid, showing normal glomeruli, bowman capsule and tubules. No significant lesion seen
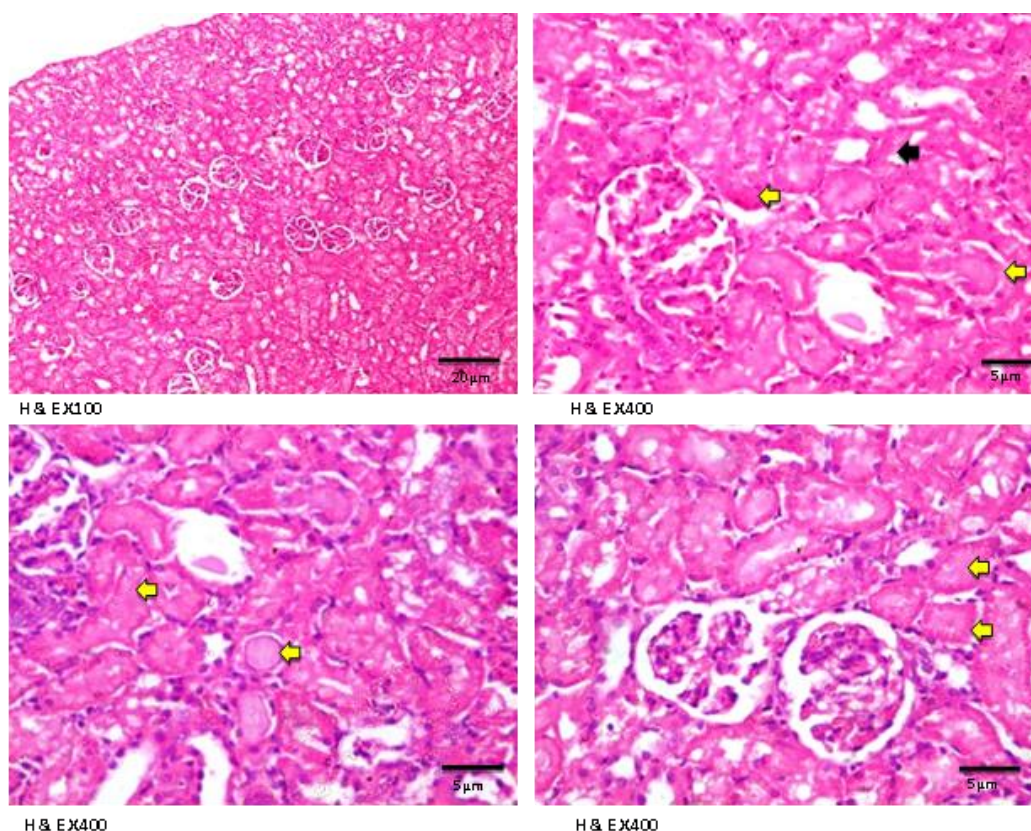
Fig 12: Photomicrographs of nephrotic tissue from rats treated with NaF and 120 mg/kg gallic acid, showing mild eosinophilic substances in the tubules (yellow arrows)

Discussion

Exposure to sodium fluoride, also known as fluorosis has been associated with acute and chronic renal damage in experimental animals and man (Chandrajith R *et al.*, 2011)

[24]. Thus, the major hallmark of the present study was the elevation of markers of kidney damage in those animals treated with NaF alone. The plasma urea was found to be higher in those animals but was corrected to some extent in

the animals that received gallic acid. Histomorphology of the NaF treated rats also showed significant inflammatory cellular infiltration in the heart and the kidneys, around the glomeruli and the tubules, with significant thinness of the tubules (Fig 7 – 12). There were also found eosinophilic proteinaceous substances in the kidney tubules, especially in the rats treated with NaF alone. Most of the lesions were however corrected in those ones that received gallic acid. The signs of acute kidney damage observed in the present study are consistent with previous reports on the effects of sodium fluoride. For example, Pain GN (2017) [1] and Rodríguez-Hernández A *et al*. (2016) [25] reported that fluoride and its products have been associated with kidney damage on dose dependent basis since medieval time because of its use in water treatment. This can even occur by indirect exposure to fetuses through the dam in-utero and through suckling (Niu R *et al*., 2016) [26].

Exposure to NaF also resulted in hypercholesterolaemia and increased HDL and LDL values in the rats. This was however, not entirely corrected by gallic acid, despite its antioxidant potentials. Previous recent observation by Pereira HA *et al*. (2016) [27] showed that fluoride use or exposure potentiates and consolidates high calorie diet induced alteration of lipid profiles in the Wistar rats. It increases plasma cholesterol, HDL and LDL, and increased oxidative stress in the affected animals, just as observed in the present study. The observed hypercholestrolaemia and disruption of lipid profile may be an indicator of hepatic damage, although, liver enzymes, ALT, AST, ALP and GGT were not determined in this study. The values of these markers of hepatic damage are expected to increase in the NaF treated rats because liver enzymes are well known markers of liver damage that has been used as diagnostic tools in confirmation of liver conditions and hepatobiliary blockage, hepatocellular carcinoma and effects of xenobiotic (Stepien M *et al*., 2016, Rathgeber S *et al*., 2017, Cuperus FJ *et al*., 2017) [28-30].

Sodium fluoride and other fluorides induce cell stress, including endoplasmic reticulum stress and oxidative stress as a result of generation of free radicals and other reactive oxygen species in the kidney, liver and other soft tissues. It also induces autophagy through c-Jun N-terminal kinase (JNK) signaling in affected cells, mitochondrial damage including cytochrome-c release, up-regulation of (uncoupling protein 2) UCP2, attenuation of ATP synthesis, and gamma H2A histone family member X (γH2AX) phosphorylation (Suzuki M *et al*., 2015) [31], a marker of DNA damage (Kuo LJ and Yang LX, 2008) [32]. In a recent study by Song C *et al*. (2017) [10], fluoride induced oxidative damage in the kidney was suggested to be an aftermath of damaging effect of NaF on Peroxisome proliferator-activated receptor-coactivator 1α (PGC-1α) and its inhibition of nuclear factor erythroid 2 (NF-E2)-related factor 2 (NRF2). Down regulation of NRF 2 subsequently results in the reduction Sirtuin 3 (Sirt3) transcription and subsequent inhibition of the activities of super oxide dismutase in the mitochondria. Excessive accumulation of ROS thereafter leads to cellular injury and death, which attracts further inflammatory cell infiltration as observed in the heart and kidney in the present study.

Gallic acid is a polyphenolic compound in grape seeds and skins (Yilmaz Y and Toledo RT, 2004) [33] with antioxidant activities against renal damage has been previously shown to mediate its antioxidant action by activating peroxisome proliferator activator receptor gamma (PPARγ) in cases of ischaemia reperfusion injury and it's associated oxidative stress in the kidneys (Singh JP *et al*., 2014) [34]. It is therefore one of the antioxidant of choice in the control or amelioration

of NaF induced renal damage due to its ability to attenuate the damaging effects of NaF on PPARγ. In the present study therefore, gallic acid at 120 mg/kg was able to attenuate some of the damaging effects of NaF on the heart and the kidney, although, the blood urea was not totally restored. This may be due to the short duration of one week for the treatment with gallic acid. Gallic acid has also been used in treatment of complications of methotrexate (Safaei F *et al*., 2017) [12] and doxorubicin (Omobowale TO *et al*., 2017) [21] toxicity in rats. Gallic acid also showed some haematinic effect as it increased PCV and RBC counts as wells differential neutrophil count in the rats treated with NaF and gallic acid. Similar activities of gallic acid on 2, 2′-Azobis(2-amidinopropane) hydrochloride (AAPH)-induced hemolysis and depletion of intracellular glutathione in erythrocytes (Ximenes VF *et al*., 2010) [35]. However, it was unable to correct the dyslipidemia that was associated NaF toxicity that was observed in the present study. Whereas, it corrected slight reduction in gonadotropins LH and FSH values to that which was comparable to those in untreated control (Table 5).

Conclusion
Sodium fluoride induced kidney damage by stimulating inflammation, which was corrected by gallic acid. The use of gallic acid also had some haematinic reaction as it increased PCV, RBC and differential neutrophil count. The use of gallic acid is therefore recommended for regular consumption, especially in areas where constant exposure to fluoride via drinking water or environmental exposure is a common occurrence.

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