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Research Article

Ameliorative Effect of Gallic Acid Against Sodium Fluoride-Induced Hypertension and Hepato-Renal Complications In Wistar Rats

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ABSTRACT

Gallic acid (GA) derivatives occur naturally in plants and it has been reported to possess antioxidant properties against various disease conditions. Here, the ameliorative effects of GA on sodium fluoride (NaF) induced-hypertension and hepatotoxicity was studied. Four groups of seven rats each were used in this study. Group A received distilled water (control), group B received NaF (300 ppm), groups C and D received NaF + GA (60 mg/kg) and NaF + GA (120 mg/kg), respectively, through oral gavage, for 7 days. The results showed that NaF alone significantly increased systolic, diastolic and mean arterial blood pressure. Administration of NaF also significantly raised both renal and hepatic hydrogen peroxide, malondialdehyde, protein carbonyl, serum myeloperoxidase and significantly decreased reduced glutathione, glutathione peroxidase, superoxide dismutase and glutathione-s-transferase when compared to the control and co-administered with GA. However, GA co-administration with NaF reduced high blood pressure and markers of oxidative stress, improved antioxidant defence system. It also ameliorated structural changes in renal and hepatic tissues. Our findings thus suggest that GA is a potential drug candidate in the treatment of NaF induced hypertension and hepatotoxicity

Keywords: *Sodium fluoride, Gallic acid, Hypertension, hepato-renal toxicity*

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INTRODUCTION

Sodium fluoride (NaF) is used globally as an important element in the prevention of tooth decay, and it has proven its effectiveness in dental caries prevention when its low level in oral hygiene materials (Clarkson *et al*., 2000). A natural source is fluoridated water in which contamination is dependent on the abundance of fluoride containing minerals in ground water (Barbier *et al*., 2010). Major side effects of fluoride toxicity include prevention of bone formation, bone brittleness and delays bone healing (World Health Organisation, 2006). Other detrimental effects associated with prolonged exposure to NaF include gastrointestinal disturbances, deranged cerebrovascular integrity and muscule wasting (de Menezes *et al*., 2003; Shivarajashankara *et al*., 2001). Further exposure to NaF causes bone remodelling, mineralization of myocardium as well as testicular necrosis. It has also been reported to cause anomalies in the chromosome thus inducing genetic damage in mammalian cells (IPCS, 2002). After oral uptake, NaF is rapidly absorbed by the intestinal tract, metabolized by the liver, distributed and excreted primarily through the urine, sweat and blood (Janssen *et al*., 1988). It has been reported to target mitochondria-rich tissues such as the kidney and liver (Barbier *et al*., 2010).

Gallic Acid (GA) is a phenolic compound that is naturally present in fruits and several plants. It has shown abilities to scavenge free radicals, scavenge inhibit oxidative stress, improve degenerative disorders (Khaledi *et al*., 2011). Its antimicrobial, hepatoprotective, cardioprotective and antifungal properties have been reported (Nayeem *et al*., 2016). GA also suppresses pro-inflammatory cytokines, reduces expression of TNF-α and IL-6 (Kim *et al*., 2006) suggesting it possesses anti-inflammatory properties.

This study was designed to investigate the probable effects of GA on NaF induced toxicities in the hepatic and renal tissues in male Wistar rats.

MATERIALS AND METHODS

Animals: Twenty-eight healthy adult male Wistar rats ranging between 139-155 g were used in this study. They were kept in the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ibadan. They were maintained in standard rat cages and given access to standard rat chow *ad libitum.*

Experimental Protocol

Group A was the Control group and was given distilled water throughout the period of the study; Group B received NaF (300 ppm) in drinking water for 7 days. Group C received NaF (300 ppm) in drinking water for 7 days concurrently with Gallic acid at 60 mg/kg by oral gavage while Group D received NaF (300 ppm) in drinking water for 7 days concurrently with Gallic acid at 120 mg/kg by oral gavage. On the 8th day, blood pressure measurements, including systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures were determined non-invasively in conscious animals by tail plethysmography using an automated blood pressure monitor (CODA S1, Kent Scientific Corporation, Connecticut, USA). Also, standard lead II electrocardiogram was recorded in rats using a 7-lead ECG machine (EDAN VE-1010, Shanghai, China). The machine was calibrated at 20 mm / mV paper speed and 50 mm/s paper speed. From the electrocardiogram, heart rate, P-wave duration, PR-interval, QRS duration, R-amplitude, QT segment and Bazett's correction of the QT interval were determined. The rats were anaesthetized with xylazine/ketamine (v/v) 0.1 ml/100 g of rats and administered intramuscularly.

Blood samples were collected via the retro-orbital venous plexus into lithium heparinized bottles and animals were sacrificed on the 9th day. Plasma was obtained for biochemical assays. Kidney and liver were excised, homogenized and centrifuged. The post mitochondrial fractions (PMF) were used for various biochemical assays. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (National Institute of Health, 1996).

Biochemical assays

Renal and hepatic hydrogen peroxide generation: This was done as described by Wolff (1994). To 50 µL of PMFs of tissues, add 100 µL of 0.1 M potassium phosphate buffer (pH 7.4), 50 µL of ammonium ferrous sulphate, 20 µL of sorbitol, 20 µL of xylenol orange (XO) and 10 µL of H_2SO4 were added. The mixture was mixed thoroughly by vortexing and a light pink colour of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 minutes. The mixtures were read at absorbance at 560 nm. The H_2O_2 generated was extrapolated from H_2O_2 standard curve.

Renal and hepatic lipid peroxidation: The Malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of the tissues according to the method Varshney and Kale (1990). 400 µL of Tris KCl, 125 µL of 30 % TCA, 100 µL of sample and 125 µL of 0.75% TBA in 0.2 M HCl was immediately added. The reaction mixture was incubated in the water bath at 80°C for 45 minutes, cooled on ice and centrifuged at 3, 000 rpm for 15 minutes. 200 µL of supernatant was taken and the absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of 1.56×10^5/M/cm.

Renal and hepatic protein carbonyl content

Protein carbonyl (PCO) contents in the tissues were measured using the method of Reznick and Packer (1994). To 100 µL of tissue supernatant were placed in glass tubes, 500 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl were added and thereafter incubated for 1 hour at room temperature. Reaction mixtures were vortexed every 15 minutes. Then, 500 µL of TCA (20%) were added and the tubes were left for 5 minutes followed by centrifugation at 4, 000 rpm for 10 minutes to collect the protein precipitates. The pellet was then washed twice with ethanol-ethyl acetate (v/v). The final precipitate was dissolved in 600 µL of 6M guanidine hydrochloride solution and incubated for 15 minutes at 37^0C. The absorbance of the sample was measured at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH ($2.2x10^4$ cm^{-1} M^{-1}) and expressed as nmoles/mg protein

Renal and hepatic GSH level: Reduced glutathione (GSH) was estimated by the method of Jollow *et al*. (1974). Briefly, 0.5 mL of 4% sulfosalicylic acid was added to 0.5 mL of sample and centrifuged at 4, 000 rpm for 5 minutes. To 0.5 mL of the resulting supernatant 4.5 mL of Ellman's reagent (0.04 g of DTNB in 100 mL of 0.1M phosphate buffer, pH 7.4 was added. The absorbance was read at 412 nm against distilled water as blank.

Renal and hepatic Vitamin C contents: Vitamin C contents were measured as described by (Jacques-Silva *et al*., 2001).

Renal and hepatic GPx activity: Glutathione peroxidase (GPx) activity was also measured according to Beutler *et al*. (1975). The reaction mixtures contain 0.5 mL of potassium phosphate buffer (pH, 7.4), 0.1 mL of Sodium azide, 0.2 mL of GSH solution, 0.1 mL of H_2O_2, 0.5 mL of sample and 0.6 mL of distilled water. The mixture was incubated in the water bath at 37°C for 5 min and 0.5 mL of TCA was added and centrifuged at 4,000 rpm for 5 min. A volume of 1 mL of the supernatant was taken and added 2 mL of K_2PHO_4 and 1 mL of Ellman's reagent. The absorbance was read at 412 nm using distilled water as blank.

Renal and hepatic SOD activity: The Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972), with slight modification (Oyagbemi *et al*., 2015). Briefly, 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. Thirty microliters of post mitochondrial fraction were added to 2.5 mL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 mL of 0.3 mM adrenaline. Increase in absorbance at 480 nm was monitored every 30 s for 150 s. One unit of SOD activity was given as

the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome

Renal and hepatic GST activity: Glutathione-S-transferase (GST) was estimated by the method of Habig *et al*. (1974) using 1-chloro-2, 4-dinitrobenzene as substrate

Renal and hepatic protein concentration: Protein concentration was determined by the Biuret method of Gornal *et al*. (1949), using bovine serum albumin (BSA) as standard and was read at 540 nm.

Plasma nitric oxide levels: The plasma nitric oxide concentrations were measured spectrophotometrically at 548 nm according to the method of Olaleye (2007).

Plasma myeloperoxidase (MPO) activity: This was determined according to the method of Xia and Zweier (1997). 10 µL of sample is mixed with 200 µL of O-dianisidine and read at 450 nm, with increases in absorbance monitored every 30 seconds for 1 minute. MPO generated is calculated as change in activity/$1.13x10^{-2}$ (µmole/L).

Plasma liver and kidney function tests: The levels of ALT (Aspartate aminotransferase); ALT (Alanine aminotransferase); ALP (Alkaline phosphatase); urea and creatinine were measured using RANDOX® laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom. All samples were analysed in triplicate, and then mean values were determined.

Histopathology: The organs (kidney and liver) tissues were immediately isolated and fixed in ten percent buffered formalin solution. They were embedded in paraffin and sectioned using a microtome, the sections were stained with haematoxylin and eosin and were observed under a light microscope. They were evaluated for structural changes (Drury, 1976).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). All results were analysed using student's t- test and one-way ANOVA followed by Tukey's post-hoc test using Graph prism 5 and p-values < 0.05 considered to be statistically significant.

RESULTS

Kidney weight increased ($p< 0.05$) significantly while the liver weight fell ($p< 0.05$) significantly in rats administered NaF alone in comparison with the control (Table 1).

There was a significant ($p< 0.05$) prolongation of both the QT and QTc intervals along increase in QRS duration in NaF alone treated rats when compared with the control and rats co-treated with GA (Table 2). The R wave amplitude was also significantly ($p< 0.05$) increased in NaF only r to that of the control (Table 2).

Table 1:
The effect of Sodium Fluoride toxicity on organ weight and relative organ weight

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
Kidney weight (g)	0.78 ± 0.07	0.86 ± 0.08 [a,]	0.94 ± 0.07[a,b]	0.88 ± 0.04[a]
Kidney/body (g/g)	0.0056 ± 0.0003	0.0056 ± 0.0005	0.0061 ± 0.0006	0.0059 ± 0.0004
Liver weight (g)	2.63 ± 0.03	2.54± 0.04[a]	2.63 ± 0.07[b]	2.67 ± 0.03[a,b]
Liver/body (g/g)	0.0191 ± 0.0026	0.0167 ± 0.0015	0.0170± 0.0007	0.0179 ± 0.0013

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Superscript ([a]) indicates significant difference at p<0.05 compared with control (Group A), while superscript ([b]) Indicates significant difference at p<0.05 compared with Group B.

Table 2:
Effect of Sodium fluoride on the electrocardiogram.

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
PR interval	37.00 ± 8.00	39.67 ± 11.37	48.67 ± 3.21	57.00 ± 2.00[a]
QRS duration	18.33 ± 0.58	19.67 ± 1.15	13.33 ± 2.08[a,b]	14.00 ± 2.65[a,b]
QT Interval	69.67 ± 9.07	64.67 ± 7.37 [a]	52.00 ± 17.78 [a, b]	61.67 ± 14.19 [b]
QT corrected	145.67 ± 19.86	123.33 ± 14.57 [a]	103.67 ± 38.37 [a, b]	120.00 ± 27.87 [b]
R wave amplitude	0.41 ± 0.02	0.68 ± 0.07[a]	0.61 ± 0.07[a]	0.42 ± 0.09[b]

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at p < 0.05. P-wave (m/s), PR interval (m/s), QRS duration (m/s), QT interval (m/s), QTC (m/s), R wave amplitude (m/s).

Table 3:
Effect of Sodium fluoride on hepatic markers of oxidative stress.

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
H_2O_2 generated	111.90±17.78	149.12±16.79[a]	125.68±16.46[a,b]	117.67±17.51[b]
MDA	3.37±0.24	6.18±0.89[a]	1.56±0.30[a,b]	1.71±0.19[a,b]
Protein Carbonyl	5.08±2.29	17.26±4.04[a]	11.78±2.87[a,b]	13.67±10.58[a]
GSH	141.68±6.58	136.36±10.63 [a]	138.71±7.62	139.15±12.26
Vitamin C	0.119±0.02	0.098 ±0.01[a]	0.102±0.02[a]	0.117±0.02[b]

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at $p < 0.05$. H_2O_2 (hydrogen peroxide generation; µmol/mg protein); MDA (malondialdehyde; µmol of MDA formed/mg protein); Protein Carbonyl (µmol /mg protein); GSH (Reduced Glutathione; µmol /mg protein); Vitamin C (µmol /mg protein).

Table 4:
Effect of Sodium fluoride on hepatic antioxidant enzymes.

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
GPx	21.70 ±2.81	20.05 ±2.62	21.80 ±1.57[b]	25.18 ±3.46[a,b]
SOD	8.57 ±0.95	7.86 ±0.88	8.48 ±0.62[b]	9.61 ±1.23[a,b]
GST	1.50 ±0.40	1.10 ±0.41[a]	1.49 ±0.69	1.31 ±0.56

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at $p < 0.05$. GPx (Glutathione Peroxidase; (units/mg protein); SOD (Superoxide Dismutase; units/mg protein); GST (Glutathione-S-transferase; mmole1- Chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein).

The administration of NaF alone significantly increased the hepatic H_2O_2 generated, MDA protein carbonyl content and significantly decreased reduced glutaythione (GSH) and Vitamin C content while coadministration of NaF and GA reduced markers of oxidative stress and improved the non-enzyme antioxidant (Table 3). The activity of hepatic GST was significantly reduced following the administration of NaF alone, however, the hepatic SOD and GPx values were reduced though not statistically significant when compared with that of the control group (Table 4). Furthermore, rats co-administered with GA (120 mg/kg) had significantly higher activities of hepatic GPx and SOD activities relative to the control and NaF alone treated rats (Table 4).

Similarly, the administration of NaF alone significantly increased the renal H_2O_2 generated, MDA, PCO content and significantly ($p< 0.05$) decreased GSH and vitamin C content, while co-administration of NaF and GA reduced markers of oxidative stress and improved the non-enzyme antioxidant defense system (Table 5). The activity of renal GST and GPx significantly reduced in NaF alone treated rats, when compared with that of the control group (Table 6) and co-administration with GA (120 mg/kg) restored the activities of renal GPx, SOD and GST activities relative to the control (Table 6). The plasma nitric oxide reduced significantly while plasma MPO increased significantly in NaF only, but the reverse was the case in rats co-administered with GA with improved NO bioavailability and significant reduction in plasma MPO activity (Table 7). The activities of ALT, AST, ALP and the plasma levels of BUN and creatinine increased significantly in NaF alone treated when compared to the control and co-treatment with GA restored the markers of liver and kidney damage to near normal values (Table 8).

Table 5:
Effect of Sodium fluoride on renal markers of oxidative stress.

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
H_2O_2	61.03±4.27	77.76±11.97[a]	72.52±11.00[a]	72.19±14.71[a]
MDA	1.93±0.06	2.18±0.34[a]	1.86±0.15[b]	2.34±0.42[a]
Protein Carbonyl	65.31±5.33	74.10±4.23[a]	52.55±15.20[a,b]	66.34±4.23[b]
GSH	79.96±9.71	72.66±9.30 [a]	72.23±7.28[a]	73.89±9.14
Vitamin C	0.50±0.05	0.47±0.06 [a]	0.62±0.09[a,b]	0.52±0.09

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at $p < 0.05$. H_2O_2 (hydrogen peroxide generation; µmol/mg protein); MDA (malondialdehyde; µmol of MDA formed/mg protein); Protein Carbonyl (µmol /mg protein); GSH (Reduced Glutathione; µmol /mg protein); Vitamin C (µmol /mg protein).

Table 6:
Effect of Sodium fluoride on renal antioxidant enzymes.

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
GPx	59.18±6.55	61.24±6.04 [a]	63.67±7.58	61.97±5.55
SOD	22.06±2.31	23.39±2.67	24.46±3.45[a]	23.57±2.15
GST	1.01±0.28	0.78±0.1[6a]	0.91±0.27 [b]	0.85±0.18

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at p < 0.05. GPx (Glutathione Peroxidase; (units/mg protein); SOD (Superoxide Dismutase; units/mg protein); GST (Glutathione-S-transferase; mmole1- Chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein).

Table 7:
Effect of Sodium fluoride on serum markers of oxidative stress.

	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
MPO	12.83±2.78	22.00±7.21[a]	17.68±4.98[a]	15.22±4.87[b]
Nitric oxide	1.60±0.19	0.96±0.11[a]	0.97±0.09[a]	0.69±0.06[a,b]

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at p < 0.05. MPO (Myeloperoxidase (µmol/minute); Nitric oxide (µmol/mg protein).

Table 8:
Effect of Sodium fluoride on liver and kidney function tests

Experiments	Group A (Control)	Group B (NaF)	Group C (NaF + Gallic acid 60 mg/kg)	Group D (NaF + Gallic acid 120 mg/kg)
AST (IU/L)	13.33±2.08	18.00±1.40 [a]	12.70±1.50	11.50±2.10
ALT (IU /L)	10.00±0.71	11.50±2.10 [a]	9.30±1.15	9.50±3.53
ALP (IU/dl)	30.5±3,.53	48.0±9.90 [a]	36.50±4.95 [a, b]	28.5±0.70 [a, b]
BUN (mmol/L)	25.67±3.51	32.00±2.00[a]	31.67±2.08 [a, b]	30.33±1.53 [a, b]
Cret. (IU/dl)	0.61±0.10	0.78±0.06 [a]	0.74±0.06 [a, b]	0.78±0.06 [a, b]

Values are presented as mean ± standard deviation. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at p < 0.05. ALT (Aspartate aminotransferase); ALT (Alanine aminotransferase); GGT (Gamma glutamyl aminotransferase); BUN (Blood urea nitrogen); Cret (Creatinine).

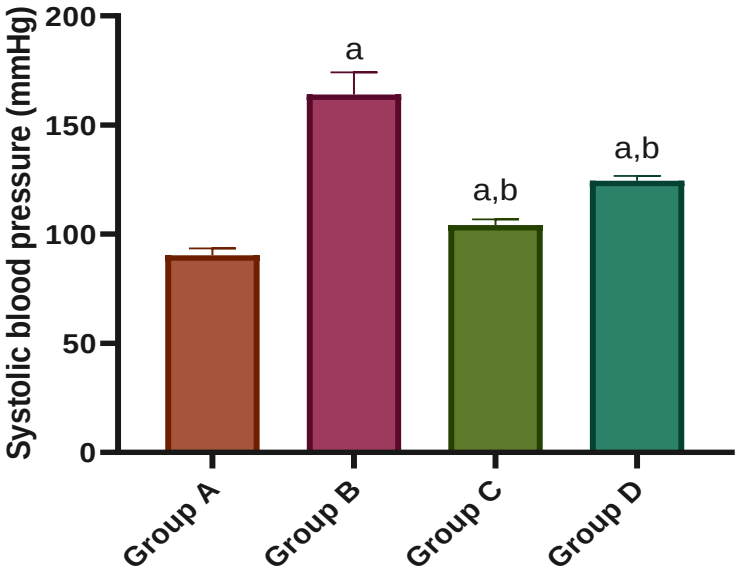

Figure 1:
The effect of Sodium fluoride on Systolic blood pressure. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at p < 0.05.

NaF alone significantly increased systolic, diastolic and mean arterial blood pressure (Figures 1-3). However, co-administration of NaF and GA (60 and 120 mg/kg) significantly reduced the systolic, diastolic and mean arterial blood pressure as indicated in Figures 1- 3. The result from this study also showed that NaF alone significantly reduced the heart rate while treatment with GA (60 and 120 mg/kg) restored the heart rate to near normal values (Figure 4). Histology of the liver shows mild infitration of the portal tract by inflammatory cells while treatment with GA ameliorated the observable histopathology (Plate 1). The administration of NaF alone led to renal epithelial degeneration and necrosis whereas mild infiltration of inflammatory cells was observed in the rats co-treated with GA (60 and 120 mg/kg) as observed in Plate 2.

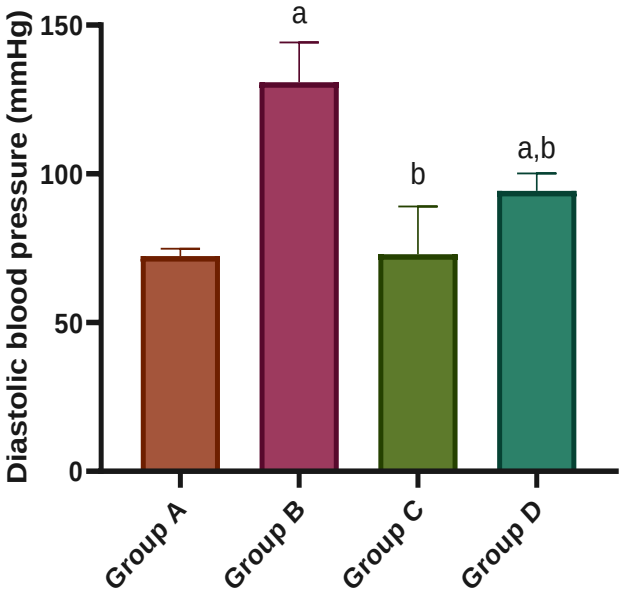

Figure 2:
The effect of Sodium fluoride on Diastolic blood pressure. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at $p < 0.05$

DISCUSSION

The results obtained from this study has shown ameliorative effect of GA on NaF induced toxicities by inhibiting the generation of reactive oxygen species (ROS) and activating both enzymatic and non-enzymatic anti-oxidant defense pathway. Excessive production of ROS leads to an imbalance in the pro-oxidant/anti-oxidant level in the body causing oxidative stress, this leads to the oxidation of large molecules, peroxidation of lipids, resulting in membrane damage and cell death, which has been incriminated in the pathogenesis of many diseases (Barbier *et al*., 2010). NaF has been described to cause deleterious effects by triggering ROS (Oyagbemi *et al.*, 2017).

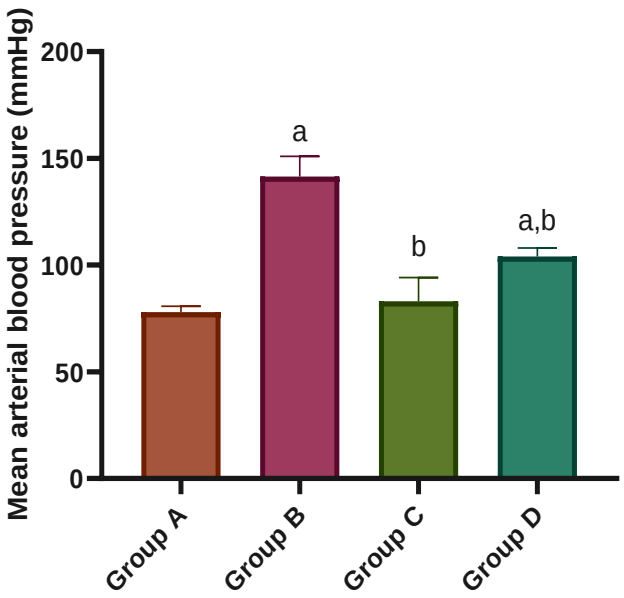

Figure 3:
The effect of Sodium fluoride on Mean arterial pressure. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at $p < 0.05$.

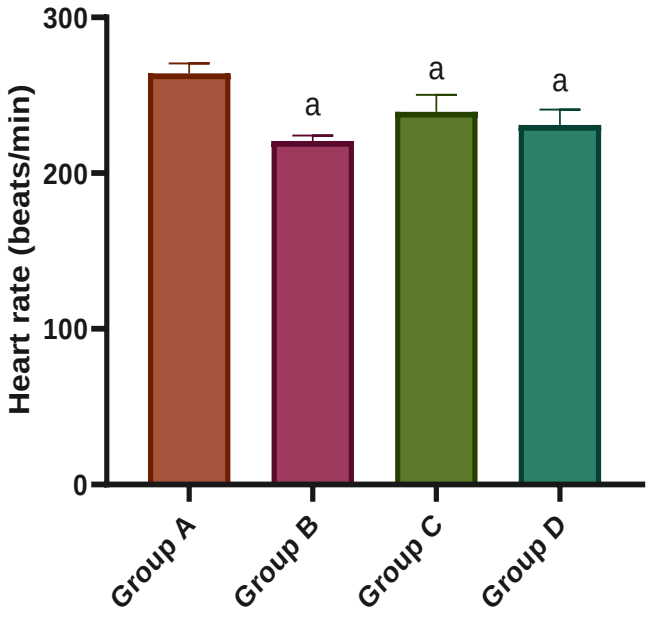

Figure 4:
The effect of Sodium fluoride on Heart rate. Group A (Control), Group B (Sodium fluoride 300 ppm), Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg). Alphabets indicates significant difference across groups at $p < 0.05$.

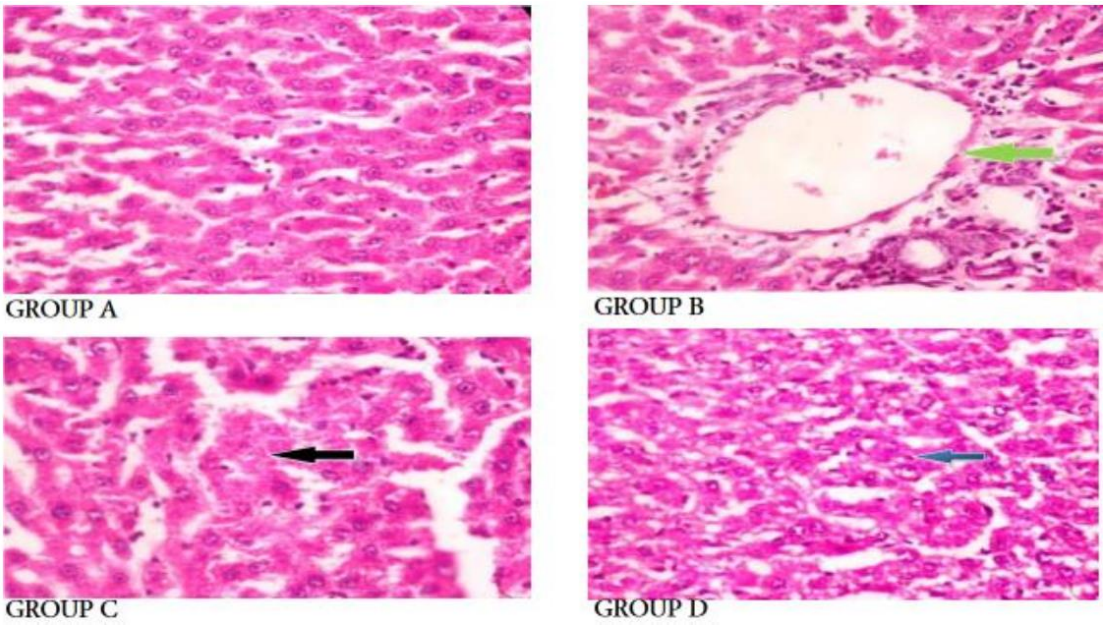

Plate 1:
Photomicrograph of the liver (X400) with H and E. Group A (Control) shows normal hepatic morphology, Group B (Sodium fluoride 300 ppm), green arrow indicates mild infitration of the portal tract by inflammatory cells, Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), black arrow shows vacuolated cytoplasm, Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg), blue arrow shows mild nuclei vesiculation

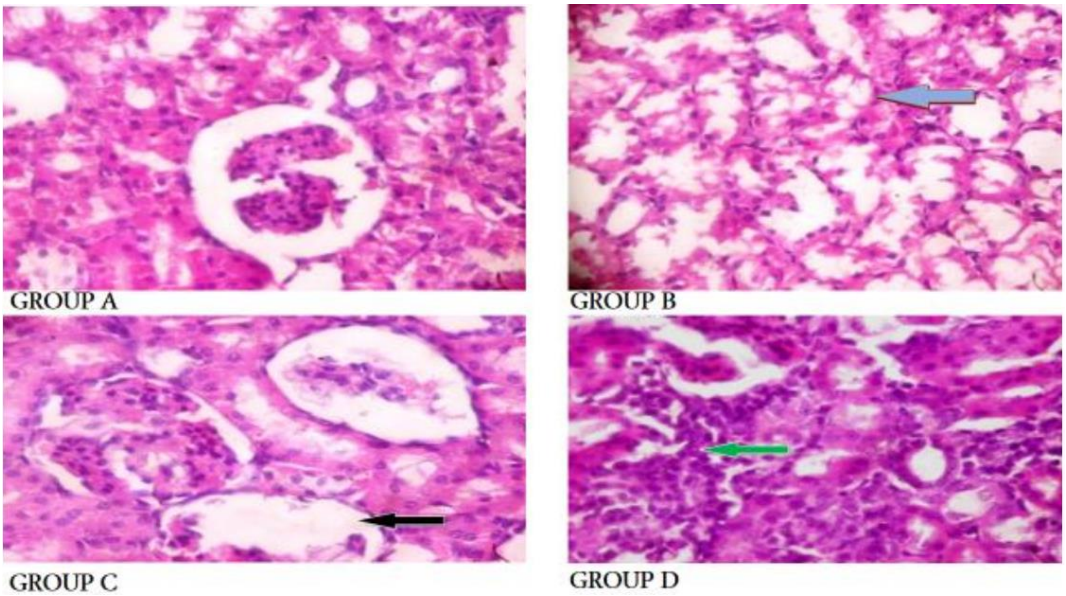

Plate 2:
Photomicrograph of the kidney (X400) with H and E. Group A (Control) shows normal kidney morphology, Group B (Sodium fluoride 300 ppm) blue arrow shows epithelial degeneration, Group C (Sodium fluoride 300 ppm + Gallic acid 60 mg/kg), black arrow shows loss of glomerular membrane, Group D (Sodium fluoride 300 ppm + Gallic acid 120 mg/kg), green arrow shows mild infiltration of inflammatory cells

In this study, there was a significant reduction in liver weight of the NaF exposed group when compared with the other groups. NaF significantly elevated hepatic and renal markers of oxidative stress (H_2O_2, MDA, PC) with a concurrent depletion in the levels of GSH, and Vitamin C. This was however significantly ameliorated by GA at the higher dose of 120 mg.kg. Oxidative stress involves lipid peroxidation, protein carbonylation and H_2O_2 generation. Lipid peroxidation generally leads to hepatic and renal toxicity (Boveris *et al*., 2008). Lipid peroxides are generally unstable and quickly decompose to other compounds including MDA (Repetto *et al*., 2012). The level of hydrogen peroxide generation is directly proportional to the MDA generation (Korchazhkina *et al*., 2003). Also, a reliable dependable

pointer of oxidative protein damage is protein carbonyl. Studies have shown that protein carbonyl levels are increased in oxidative stress (Fernando *et al*., 2016), we therefore demonstrate that NaF induces oxidation of proteins resulting in the production of protein carbonyl and that GA acts by mitigating protein oxidation. Superoxide radical is converted to H_2O_2, a process catalysed by superoxide dismutase, and the hydrogen peroxide generated oxidative stress. The H_2O_2 also raises the concentration of superoxide anion in the cells through NOS and NADPH synthase (Coyle *et al*., 2006), thus, aggravating oxidative stress. Vitamin C is a water soluble antioxidant that acts by scavenging oxygen free radicals generated in oxidative processes. A previous study has reported that GA improved renal function by restoring its tissue antioxidant levels (Veerapur *et al*., 2011).

Hepatic and renal antioxidant enzymes (GPx and GST) were significantly depleted in the NaF alone treated group while GA induced improvement to levels comparable to the control group. The SOD dismutates superoxide radical into H_2O_2, this is further detoxified by reduced glutathione peroxidase (GPx) and catalase (CAT). In our study, however, we saw a marginal but not significant increase in the renal SOD levels in the NaF exposed group, this could be explained as an adaptive initial defence mechanism in which the excess production of ROS triggers the body to produce anti-oxidant enzymes in a bid to counteract the deleterious effects of the ROS generated. Generation of ROS might have also contributed significantly to the observed shortened QT and QTc, respectively (Lazzerini *et al.*, 2015). This finding is quite similar to an earlier report by Kale *et al*., (1999) and Chlubek *et al*., (2003) who reported significant increases in SOD levels following ROS generation. The Glutathione-S-transferases are essential antioxidant enzymes, functions in removing hydrogen peroxide and also conjugate lipid oxidation products. Glutathione peroxidases on the other hand performs the role of reducing peroxides and other hydroperoxides (Patekar *et al*., 2013). The deficiencies in these antioxidants and enzymes may further aggravate the effects of reactive oxygen species (Gospodaryov & Lushchak, 2012)

Likewise, in this study, we discovered a significant elevation in serum MPO levels in the NaF alone treated group together with depletions in the GA treated groups at both doses. The MPO is released by the neutrophils and macrophages in inflammation, it is also involved in the production of hypochlorite from chloride and hydrogen peroxide, with reported increase levels in inflammation, oxidative stress and cardiac damage (Loria *et al*., 2008). Our result suggests that NaF caused hypertension, renal and hepatic damage was through inflammation and oxidative stress. This also confirms that GA possesses anti-inflammatory and antioxidant properties. GA has earlier been reported to inhibit inflammation by repressing proinflammatory cytokines and cyclooxygenase-2 (Jung *et al*., 2011).

Nitric oxide (NO) levels were depleted in the NaF alone treated group, however GA ameliorated this depletion by improving the NO bioavailability. NO regulates vascular tone and blood pressure, and its reduced levels has been linked with increased blood pressure (Klahr, 2001), thus, making it an important mediator of hypertension. It causes vasodilation, inhibits platelet aggregation and also exerts anti-inflammatory properties. NaF in this study induced depletions of NO levels, thus, resulting in hypertension. Increased MPO has also been reported to reduce the bioavailability of NO (Loria *et al*., 2008). Therefore, the increase in myeloperoxidase levels by NaF might also contribute to the depletions in NO levels. Blood pressure parameters (SBP, DBP and MAP) were pointedly elevated in the NaF alone treated group; while GA treated groups improved the levels (Figures 1, 2 and 3). Previous studies have shown that NaF could cause elevated blood pressure levels (Jin *et al*., 2017). Jin *et al*., (2017) stated that GA reduces blood pressure in spontaneously hypertensive rats; our results corroborated their findings.

Correspondingly, histopathological findings revealed that NaF alone exposed group exhibited mild inflammatory lesions on the liver and epithelial degeneration on the kidney, however with GA at both doses, we observed milder lesions than those shown by the NaF alone group. Fluoride induces the generation of ROS leading to oxidative stress, by peroxidation of lipids and protein carbonation. Studies have shown that its toxicity can lead to cell death, apoptosis and/or necrosis both *in vivo* and *in vitro*. Necrosis has been observed as a primary mechanism of cell death in the presence of relatively high fluoride concentrations (Barbier *et al*., 2010). In another experiment, the administration of NaF alone significantly increased markers of liver and kidney damage as indicated by significant increases in AST, ALT, ALP, BUN and creatinine. The release of high levels of AST, ALT, ALP into the blood has been linked to hepatic damage due to the rupture of the hepatocyte membrane (Yang *et al*., 2018; Fernández-Martínez *et al*., 2018; Xueting *et al*., 2018). The ability of GA to the high level of these markers of liver damage is indicative of hepatoprotective effect of GA. Previous evidence has documented the hepatoprotective effect of GA (Sachdeva *et al*., 2015; Karimi-Khouzani *et al*., 2017). The increase in serum BUN and creatinine levels is an indication of renal damage (Purena *et al*., 2018; Huang *et al*., 2018). The increase in BUN and creatinine might also be associated with hypertension-induced renal damage (Nunes *et al*., 2018; Abdel-Zaher *et al*., 2018). This study also confirmed the report on protective effect of GA against NaF-induced nephrotoxicity (Nabavi *et al*., 2013). Therefore, this study demonstrates that GA could be used to ameliorate hepatoxicity and nephrotoxicity associated with NaF-induced hypertension.

In conclusion, we have established that gallic acid protected against NaF induced hypertension and hepato-renal toxicity by lowering blood pressure, inhibiting lipid peroxidation, protein carbonylation, and by improving both enzymatic and non-enzymatic antioxidant pathway thus making it a promising compound for drug development in the treatment of sodium fluoride induced hypertension and hepatic damage.

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