Research Communication

# Quercetin attenuates hypertension induced by sodium fluoride via reduction in oxidative stress and modulation of HSP 70/ERK/PPARγ signaling pathways


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## Abstract

Hypertension is one of the silent killers in the world with high mortality and morbidity. The exposure of humans and animals to fluoride and/or fluoride containing compounds is almost inevitable. This study investigated the modulatory effects of quercetin on sodium fluoride (NaF)-induced hypertension and cardiovascular complications. Forty male rats were randomly separated into four groups ($n$ =10). Group A animals served as the control, rats in Group B were exposed to 300 ppm of NaF, Groups C and D animals were exposed to 300 ppm of NaF along with quercetin orally at 50 mg/kg and 100 mg/kg orally by gavage, while NaF was administered in drinking water, respectively, for a week. Administration of NaF caused severe hypertension as indicated with significant increases in the systolic, diastolic, and mean arterial blood pressure, together


***Abbreviations:*** *ANOVA, one-way analysis of variance; AOPP, advanced oxidation protein product; AP-1, activator protein-1; AST, aspartate aminotransferase; CAT, catalase; CDNB, 1,2 dichloro 4-nitrobenzene; Cyt C, cytochrome CDAB, diaminobenzidine; DBP, diastolic blood pressure; ECG, electrocardiogram; ERK1/2, extracellular signal regulated kinase 1/2; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione-S-transferase; $H_2O_2$, hydrogen peroxide generation; HRP, horse radish peroxidase; HSP70, heat shock protein70; I/R, ischemia/reperfusion; JNK, c-Jun NH2-terminal kinase; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MNPCE, micronucleated polychromatic erythrocytes; MPO, myeloperoxidase; NaF, sodium fluoride; NF-kB, nuclear factor kappa B; NO, nitric oxide; PPARγ, peroxisome proliferator-activated receptor-gamma; ROS, reactive oxygen species; SBP, diastolic blood pressure; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloro acetic acid*




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with prolonged ventricular depolarization (QRS) and the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle (QT) intervals when compared with controls. NaF significantly decreased the activities of antioxidant enzymes, caused increase in markers of oxidative stress and renal damage when compared with controls. Immunohistochemical staining revealed lower expressions of Hsp70, ERK, and PPARγ in the heart, kidney, and aorta of rats-administered NaF relative to the controls. Together, quercetin co-treatment with NaF restored blood pressure, normalized QRS interval, and improved antioxidant defense system. © 2018 BioFactors, 00(00):1–15, 2018

***Keywords:*** *sodium fluoride; hypertension; quercetin; oxidative stress; nutraceutical*

## 1. Introduction

The exposure of humans and animals to fluoride and/or fluoride-containing compounds is almost inevitable because virtually all foodstuffs and vegetation contain at least traces of fluorine or fluoride [1]. In addition, fluoride is used in household chemicals such as insecticides and rodenticides and is widely used in toothpaste and mouth rinses (as sodium monofluorophosphate) for elimination of acid-producing oral bacteria [2,3]. Fluoride intoxication may occur through drinking water, food, or industrial sources and fluoride abuse in dental care has been reported to cuase several acute and chronic health problems [4,5]. Deleterious effects such as dental and skeletal fluorosis, increased rates of bone fractures, decreased birth rates, increased rates of urolithiasis, impaired thyroid function, and lower intelligence in children have **also** been reported [6]. Also, fluoride exposure has been associated with mineralization of the myocardium and testicular degeneration [7].

Fluoride is known to induce oxidative stress in mammalian tissues, alter gene expression, and influences distinct signaling pathways involved in proliferation and apoptosis such as the mitogen-activated protein kinase (MAPK), activator protein-1 (AP-1), and nuclear factor kappa B (NF-kB) pathways [8,9]. Although, several mechanisms have been suggested for fluoride toxicity, the induction of oxidative stress, in recent times, has become recognized as a central mechanism of fluoride-induced organ specific pathologies [10]. Fluoride aggravates biochemical stress in mammalian tissues by generating imbalance between reactive oxygen species (ROS) and systemic antioxidants thereby inducing oxidative stress and inhibiting several groups of enzymes including enolase, phosphatases, catalase (CAT), and peroxidase [11]. Consequently, several studies, particularly in the last few decades, have investigated and documented the protective effects of orally active antioxidants on fluoride-induced toxicity.

Quercetin (3,3′,4′,5,7-pentahydroxy-2-phenylchro-men-4-one) is an orally active bioflavonoid antioxidant that has been reported to play critical roles in reducing cardiovascular diseases [12]. The powerful antioxidant properties of quercetin, reported to be several times higher than that of glutathione and vitamin E, are attributable to its constituent catechol and hydroxyl groups in the molecule [13,14]. Quercetin has been documented to mitigate oxidative stress associated with various toxic compounds and scavenges ROS in mammalian tissues [15]. Nabavi et al. [4] reported cardioprotective effect for quercetin in NaF-induced oxidative stress. Also, increased bioavailability of exogenous nitric oxide (NO) in the *Corpus cavernosum* of mice following quercetin exposure has been reported by Ertuğ et al. [4]. Since exposure to fluoride either through fluoridated products or contaminated food and water continues to increase, identifying possible preventive, curative, and therapeutic possibilities have become imperative [16]. This study was designed to investigate the protective effects of quercetin on acute fluoride-induced hypertension and cardiac complications. However, this study aims at studying the molecular mechanism of action of NaF-induced hypertension and endothelial dysfunction. This seeks to report for the first time on the influence of NaF intoxication on Hsp70, ERK, and PPARγ signaling pathways.

## 2. Materials and methods

### 2.1. Chemicals

Quercetin, Thiobarbituric acid (TBA), 1,2-dichloro-4-nitrobenzene, trichloro acetic acid (TCA), Sodium Fluoride (NaF), sodium hydroxide, xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), O-dianisidine, and hydrogen peroxide ($H_2O_2$) were purchased from Sigma, St. Louis, MO. Normal goat serum, Biotinylated antibody, and horse radish peroxidase (HRP) System was purchased from KPL, Inc., Gaithersburg, MD. Cytochrome C (Cyt C), Hsp70, p38, ERK, and rabbit anti-PPARγ antibodies were purchased from Bioss Inc., Woburn, MA. Diaminobenzidine (DAB) tablets were purchased from AMRESCO LLC, Cleveland, OH. All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK). All other chemicals were of analytical grade.

### 2.2. Experimental animals and study design

Forty male rats aged 8 weeks of the Wistar strain weighing 120–142 g obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were used for this study. The rats were randomly divided into four groups with 10 rats per group. Group A served as the control group was given water, Group B was exposed to 300 ppm (300 mg/L) of NaF in drinking water for a week, Groups C and D were exposed to 300 ppm (300 mg/L) of NaF and concurrently administered quercetin by oral gavage daily at a dosage of 50 and 100 mg/kg respectively for a week. The rats were kept

in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow *ad libitum*. All the animals received humane care according to the criteria outlined in the Public Health Service Policy on Humane Care and the Use of Laboratory Animals [17].

### 2.3. Blood pressure measurement

Twenty-four hours after the last administration of NaF, blood pressure parameters, 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, Torrington, CT). The average of at least nine readings, were taken in the quiescent state, following acclimatization, was recorded per animal on the eighth day of the experiment.

### 2.4. Electrocardiography

Standard lead II electrocardiogram (ECG) was recorded in conscious 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 ECG, parameters such as 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 anesthetized with xylazine/ketamine (v/v) 0.1 ml/100 g of rats and administered intramuscularly.

### 2.5. Blood sample collection and serum preparation

Approximately 3 mL of blood were collected into plain bottles, by retro–orbital venous puncture using plain tubes and allowed to clot. The clotted blood was then centrifuged at 4000 revolutions per minute (rpm) for 10 min. Clear serum was separated with Pasteur pipette into another plain tube and stored at 4 °C until the time of analysis.

### 2.6. Preparation of cardiac and renal homogenates

The organs (kidney and hearts) were excised, rinsed and homogenized using 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% KCl. The homogenates were subjected to cold centrifugation at 4 °C using a speed of 10,000 rpm for 15 minutes. The post mitochondrial fractions (PMFs) obtained from cardiac and renal homogenates were used for biochemical assays.

## 3. Biochemical assays

### 3.1. Cardiac and renal antioxidant defense system

The GSH content was estimated by the method of Jollow et al. [18]. The protein and non-protein thiol contents were determined as described by Ellman [19]. The activity of superoxide dismutase (SOD) was carried out by the method of Misra and Fridovich [20], with slight modification (Oyagbemi et al. [21]. The CAT activity was determined according to the method of Shinha [22]. Glutathione peroxidase (GPx) activity was also measured according to Beutler et al. [23]. Glutathione-S-transferase (GST) was estimated by the method of Habig et al. [24]. Protein concentration was determined by the method of Gornal *et al*. [25], using bovine serum albumin as standard.

### 3.2. Determination of serum markers of inflammation, hypertension, and renal damage

Advanced oxidation protein product (AOPP) contents were determined as described by Kayali et al. [26]. The serum myeloperoxidase (MPO) activity was determined according to the method of Xia and Zweier [27]. The activity of xanthine oxidase was determined according to method of Akaike et al. [28]. The serum **NO** concentrations were measured according to the method of Olaleye et al. [29]. The blood urea nitrogen (BUN), creatinine, and aspartate aminotransaminase (AST) levels were determined using Randox kits (Crumlin BT29 4QY, UK) according to manufacturer's instructions.

### 3.3. Cardiac and renal makers of oxidative stress

Hydrogen peroxide generation ($H_2O_2$) level was determined according to the method of Wolff [30]. The malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of cardiac and renal tissue according to the method Varshney and Kale with a molar extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$ [31].

### 3.4. Histopathology

Small pieces of kidney, heart, and aorta were fixed in 10% formalin, embedded in paraffin wax, and sections of 5–6 mm in



**TABLE 1** ***Protective effect of Quercetin on blood pressure and haemodynamic parameters in Fluoride-induced non anaesthesised rats***

| *Parameter* | *Control* | *NaF* | *NaF + Quercetin 50 mg/kg* | *NaF + Quercetin 100 mg/kg* |
|---|---|---|---|---|
| SBP (mm Hg) | 119.43 ± 2.57 | 172.14 ± 3.02[a] | 139.85 ± 3.93[a, b] | 140.14 ± 2.60[a, b] |
| DBP (mm Hg) | 87.28 ± 5.21 | 140.42 ± 3.55[a] | 105.57 ± 4.27 [a, b] | 98.28 ± 3.30 [b] |
| MAP (mm Hg) | 97.71 ± 3.81 | 150.71 ± 3.03[a] | 116.85 ± 389 | 111.85 ± 2.19 |

*Values are presented as mean ± standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A), while superscript (@) Indicates significant difference at P < 0.05 compared with Group B.*
*DBS, diastolic blood pressure (mm Hg); MAP, mean arterial blood pressure (mm Hg); SBP, Systolic blood pressure (mm Hg).*

**TABLE 2** ***The effect of NaF toxicity on organ weight and relative organ weight***

| | *Group A (Control)* | *Group B (NaF)* | *Group C (NaF + Quercetin 50 mg/kg)* | *Group D (NaF + Quercetin 100 mg/kg)* |
|---|---|---|---|---|
| Heart weight (g) | 0.48 ± 0.064 | 0.030 ± 0.0053* | 0.35 ± 0.024* @ | 0.39 ± 0.069*@ |
| Heart/ Body (g/g) | 0.0051 ± 0.004 | 0.0066 ± 0.0063 | 0.0063 ± 0.0041 | 0.0060 ± 0.0047 |
| Kidney weight (g) | 0.72 ± 0.075 | 0.53 ± 0.11* | 0.62 ± 0.043*@ | 0.71 ± 0.081@ |
| Kidney/body (g/g) | 0.0034 ± 0.0034 | 0.0037 ± 0.0031 | 0.0036 ± 0.0024 | 0.0033 ± 0.0039 |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at P < 0.05 compared with control (Group A), while superscript (@) Indicates significant difference at P < 0.05 compared with Group B.*

**TABLE 3** ***The effect of NaF on serum markers of renal and cardiac damage***

| *Serum* | *UREA* | *CREAT* | *AST* |
|---|---|---|---|
| Control | 2.38 ± 0.11 | 2.93 ± 0.84 | 14.84 ± 1.62 |
| Group B (NaF) | 2.80 ± 1.40 | 3.61 ± 0.27* | 17.97 ± 1.22* |
| Group C (NaF + Quercetin 50 mg/kg) | 1.97 ± 0.84 | 2.56 ± 1.05 | 13.58 ± 1.36@ |
| Group D (NaF + Quercetin 100 mg/kg) | 1.68 ± 0.40* | 2.93 ± 0.95 | 12.97 ± 1.20*@ |

*Values are presented as mean ± standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at (P < 0.05) compared with control (Group A), superscript (@) indicates significant difference at (P < 0.05) to compared with Group B in each column. Urea (μmol/L), Crea (creatinine; μmol/L), AST (Aspartate aminotransferase; μmol/L)*

**TABLE 4** ***The effect of NaF on cardiac nonenzymatic antioxidants***

| *Heart* | *GSH* | *PT* | *NPT* |
|---|---|---|---|
| Group A (Control) | 73.61 ±3.19 | 22.35 ±0.87 | 32.62 ±3.13 |
| Group B (NaF) | 58.73 ±3.31* | 26.43 ±3.42 | 36.67 ±9.19 |
| Group C (NaF + Quercetin 50 mg/kg) | 66.06 ±4.73 | 23.67 ±2.86 | 29.06 ±0.43 |
| Group D (NaF + Quercetin 100 mg/kg) | 60.08 ± 4.73* | 15.67 ± 0.84*@ | 27.63 ± 0.67* |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at P < 0.05 compared with control (Group A), Superscript (@) indicates significant difference at P < 0.05 to compared with Group B.*
*GSH, reduced glutathione (micromole/mg protein); NPT, non-protein thiol (nmole/mg protein); PT, protein thiol (nmole/mg protein).*

**TABLE 5** ***The effect of NaF on renal non-enzymatic antioxidants***

| *Kidney* | *GSH* | *NPT* | *PT* |
|---|---|---|---|
| Group A (Control) | 105.36 ±10.24 | 89.86 ±15.14 | 52.40 ±8.67 |
| Group B (NaF) | 98.32 ±6.63 | 99.92 ±14.28 | 56.04 ±3.10 |
| Group C (NaF + Quercetin 50 mg/kg) | 100.89 ±7.22 | 89.36 ±4.03 | 53.83 ±5.37 |
| Group D (NaF + Quercetin 100 mg/kg) | 100.23 ±11.16 | 86.36 ±6.10 | 51.34 ±19.47 |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg).*
*GSH, reduced glutathione (micromole/mg protein); PT, protein thiol (nmole/mg protein); NPT, non-protein thiol (nmole/mg protein).*

**TABLE 6** ***The effect of NaF on cardiac antioxidant enzymes***

| *Heart* | *SOD* | *CAT* | *GST* | *GPx* |
|---|---|---|---|---|
| Group A (Control) | 18.39 ± 1.59 | 65.93 ± 4.08 | 5.71 ± 0.58 | 96.94 ± 3.50 |
| Group B (NaF alone) | 12.82 ± 3.40* | 51.48 ± 3.17* | 2.21 ± 0.70* | 79.32 ± 1.11* |
| Group C (NaF + Quercetin 50 mg/kg) | 15.50 ± 0.24* | 56.89 ± 6.80* | 4.99 ± 1.07$^{@}$ | 84.72 ± 10.33$^{@}$ |
| Group D (Naf + Quercetin 100 mg/kg) | 15.73 ± 2.68 | 51.59 ± 0.61* | 4.22 ± 0.85* | 87.69 ± 1.31$^{@}$ |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at P < 0.05 compared with control (Group A), superscript (@) indicates significant difference at P < 0.05 to compared with Group B.*
*CAT, catalase (mmole $H_2O_2$ consumed/min/mg protein); GPx, glutathione peroxidase (units/mg protein); GST, glutathione-S-transferase (mmole1), Chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein; SOD, superoxide dismutase (units/mg protein).*

**TABLE 7** ***The effect of NaF on renal antioxidant enzymes***

| *Kidney* | *SOD* | *CAT* | *GST* | *GPx* |
|---|---|---|---|---|
| Group A (Control) | 27.13 ± 4.74 | 34.17 ± 12.54 | 34.17 ± 12.54 | 258.42 ± 19.42 |
| Group B (NaF Only) | 21.43 ± 0.73* | 19.86 ± 0.72* | 19.86 ± 0.72* | 228.52 ± 8.76* |
| Group C (NaF + Quercetin 50 mg/kg) | 23.64 ± 1.92$^{@}$ | 28.28 ± 4.27$^{@}$ | 28.28 ± 4.27$^{@}$ | 239.27 ± 6.97 |
| Group D (NaF + Quercetin 100 mg/kg) | 24.39 ± 2.69$^{@}$ | 31.08 ± 2.48$^{@}$ | 31.08 ± 4.28$^{@}$ | 241.40 ± 24.25 |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at P < 0.05 compared with control (Group A), Superscript ($^{@}$) indicates significant difference at P < 0.05 to compared with Group B. CAT, catalase (mmole $H_2O_2$ consumed/min/mg protein); GPx, glutathione peroxidase (units/mg protein); GST, glutathione-S-transferase; mmole CDNB-GSH complex formed/min/mg protein; SOD, superoxide dismutase (units/mg protein).*

**TABLE 8** ***The effect of NaF on cardiac markers of oxidative stress***

| *Heart* | *MDA* | *$H_2O_2$* | *AOPP* |
|---|---|---|---|
| Group A (Control) | 1.40 ± 0.41 | 13.59 ± 1.33 | 34.51 ± 2.12 |
| Group B (NaF) | 2.12 ± 0.28* | 15.37 ± 2.33* | 44.75 ± 2.89* |
| Group C (NaF + Quercetin 50 mg/kg) | 1.88 ± 0.39 | 13.07 ± 2.13 | 34.83 ± 4.80@ |
| Group D (NaF + Quercetin 100 mg/kg) | 1.82 ± 0.61 | 13.29 ± 0.67 | 33.42 ± 0.22@ |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at P < 0.05 compared with control (Group A), @ Indicates significant difference at P < 0.05 to compared with Group B.*
*AOPP, advanced oxidation protein product (nmoles/mg protein); $H_2O_2$, hydrogen peroxide (μmol/mg protein); MDA, malondialdehyde (μmol/mg protein); XO, xanthine oxidase (units/min/mg protein).*

**TABLE 9** ***The effect of NaF on renal markers of oxidative stress***

| *Kidney* | *MDA* | *$H_2O_2$* | *AOPP* |
|---|---|---|---|
| Group A (Control) | 3.12 ± 0.63 | 26.46 ± 2.47 | 82.64 ± 12.06 |
| Group B (NaF) | 6.94 ± 0.47* | 36.63 ± 2.24* | 119.76 ± 12.29* |
| Group C (NaF + Quercetin 50 mg/kg) | 5.88 ± 0.46*@ | 32.39 ± 4.87*@ | 96.44 ± 18.94@ |
| Group D (NaF + Quercetin 100 mg/kg) | 4.78 ± 0.96*@ | 31.12 ± 5.14@ | 92.00 ± 15.93@ |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Superscript (*) indicates significant difference at P < 0.05 compared with control (Group A), Superscript (@) indicates significant difference at P < 0.05 to compared with Group B. AOPP, advanced oxidation protein product (nmoles/mg protein); MDA, malondialdehyde (μmol of MDA formed/mg protein); $H_2O_2$, hydrogen peroxide (μmol/mg protein).*

**TABLE 10** ***The effect of NaF toxicity on electrocardiographic (ECG) changes***

| | *Heart Rate (beats/min)* | *P-wave (m/s)* | *QRS (m/s)* | *QT interval (m/s)* | *QTC (m/s)* |
|---|---|---|---|---|---|
| Group A (Control) | 243.33 ± 14.64 | 22.75 ± 2.22 | 11 ± 0.82 | 68.00 ± 12.62 | 0.48 ± 0.086 |
| Group B (NaF) | 349.33 ± 10.41* | 21.00 ± 2.65 | 16.33 ± 3.51* | 74.67 ± 10.69* | 0.47 ± 0.10 |
| Group C (NaF + Quercetin 50 mg/kg) | 290.5 ± 11.21*@ | 19.33 ± 1.15* | 14.5 ± 3.0* | 71.5 ± 14.11 | 0.67 ± 0.13* |
| Group D (NaF + Quercetin 100 mg/kg) | 281.33 ± 7.64*@ | 20.00 ± 1.41 | 11 ± 1.73@ | 65.5 ± 4.95 | 0.48 ± 0.078 |

*Values are presented as mean ±standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg).*
*Group A- Control; Group B- NaF (300 ppm); Group C- NaF + quercetin (50 mg/kg); Group D- NaF + Quercetin (100 mg/kg). Values are presented as mean ±standard deviation. * Indicates significant difference at P < 0.05 compared with control (Group A), @ Indicates significant difference at P < 0.05 to compared with Group B.*

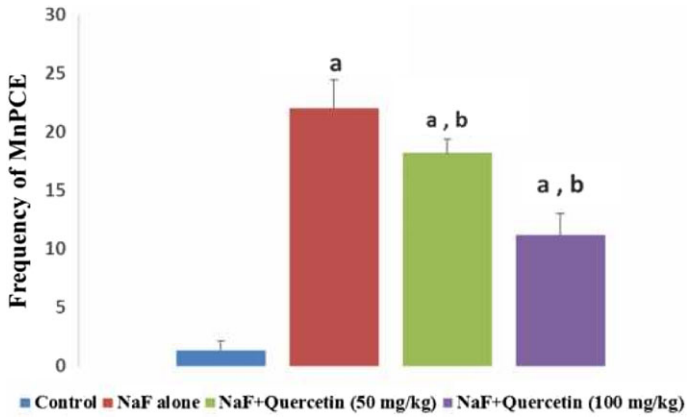


**FIG 1**

*The effect of NaF on micronucleated polychromatic erythrocyte. Values are presented as mean ± standard deviation (n = 10). Group A (Control), Group B (NaF 300 ppm), Group C (NaF 300 ppm + quercetin 50 mg/kg), Group D (NaF 300 ppm + quercetin 100 mg/kg). Group A: Control; Group B: NaF (300 ppm); Group C: NaF + quercetin (50 mg/kg); Group D: NaF + quercetin (100 mg/kg). Micronucleated polychromatic erythrocyte (MnPCE).Group A: Control; Group B: NaF (300 ppm); Group C: NaF + quercetin (50 mg/kg); Group D: Nat' + quercetin ( IOU mg/kg). Values are presented as mean ± standard deviation. *Indicates significant difference at P < 0.05 compared with control (Group A), @ Indicates significant difference at P < 0.05 to compared with Group B.*

thickness and thereafter stained with Hematoxylin and Eosin (H&E) for histopathological examination according to the methods described by Drury et al. [32]. Thereafter, the sections were examined with light microscope.

### 3.5. Genotoxicity

This was done using *in vivo* micronucleus assay technique as described previously [33]. The proximal ends of the femurs were carefully removed with a pair of scissors until a small opening to the marrow became visible. The femur was submerged in fetal calf serum and the marrow was flushed out gently by aspiration and flushing on glass slides. The marrow suspension was positioned on one end of a slide and spread by pulling the material behind polished cover glass held at an angle of 45°. Slides were fixed in methanol for 3–5 min; allowed to dry for 24 h and later stained with May–Gruenwald and later with 5% diluted Giemsa solution for at least 30 min. Slides were then rinsed in phosphate buffer for about 30 sec and in distilled water. Slides were then air-dried. The dried stained slides were then mounted in a mixture distyrene, plasticizer and xylene DPX with coverslips. They were later viewed under the microscope at ×100 magnification using oil immersion for the presence of micronucleated polychromatic erythrocytes (MNPCE). Scoring was done using a tally counter.

### 3.6. Immunohistochemical staining for Hsp70, Cyt C, p38, ERK, and PPARγ expressions

The immunohistochemistry was described as reported by Oyagbemi et al. [33,34]. To determine the expression of Hsp70, Cyt C, p38, ERK, and rabbit anti-PPARγ in the heart, kidney, and aorta, fixed tissues were embedded in paraffin and sectioned at a thickness of 5 μm. The sections were subsequently deparaffinized in xylene and rehydrated with graded alcohol. Antigen retrieval was carried out by immersing the slides in 10 mM citrate buffer at 95–100 °C for 25 min with subsequent peroxidase quenching in 3% $H_2O_2$/methanol solution. The sections were blocked in goat serum followed by an overnight incubation at 4 °C in the goat anti-rabbit Hsp70, Cyt C, p38, ERK, and PPARγ primary antibodies (1: 200). Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 μg/mL, 1: 1000) secondary antibody and subsequently, streptavidin peroxidase (HRP–streptavidin) according to manufacturer's protocol (HistoMark®, KPL, Gaithersburg, MD). Reaction product was enhanced with DAB (Amresco®, Solon, OH, 44139, USA) for 6–10 min and counterstained with high definition hematoxylin (Enzo®, NY), with subsequent dehydration in ethanol. The sections were subsequently dehydrated in ethanol, cleared in xylene. The slides were covered with coverslips and sealed with resinous solution. The immunoreactive positive expression of Hsp70, Cyt C, p38, ERK, and PPARγ antirabbit intensive regions were viewed starting from low magnification on each slice then with 400× magnifications using a photo microscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

### 3.7. Statistical analysis

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

## 4. Results

### 4.1. Blood pressure parameters, organ weight, and serum markers of organ damage

In this study, rats administered **NaF** alone showed severe hypertension with an approximate 50 mm Hg increase in the systolic, diastolic, and mean arterial blood pressure parameters compared with the control. These increases in blood pressure were significantly attenuated by quercetin co-administration with NaF but were higher than that of the control (Table 1). The mean arterial blood pressure was not significantly altered by quercetin co-treatment (Table 1). There was a significant ($P < 0.05$) decrease in the heart weight of NaF alone treated rats when compared with the Control, while the heart weight of rats co-administered with quercetin (50 and 100 mg/kg) increased significantly when compared with NaF-alone group. Also, there was a significant ($P < 0.05$) decrease in the Kidney weight of Groups NaF alone when compared with Control, while the reduction in the kidney weights was reversed in rats co-administered with quercetin (50 and 100 mg/kg) (Table 2). The serum blood urea nitrogen level was unaltered and that quercetin only decreased the activity of aspartate aminotransferase (AST) following administration of NaF (Table 3).

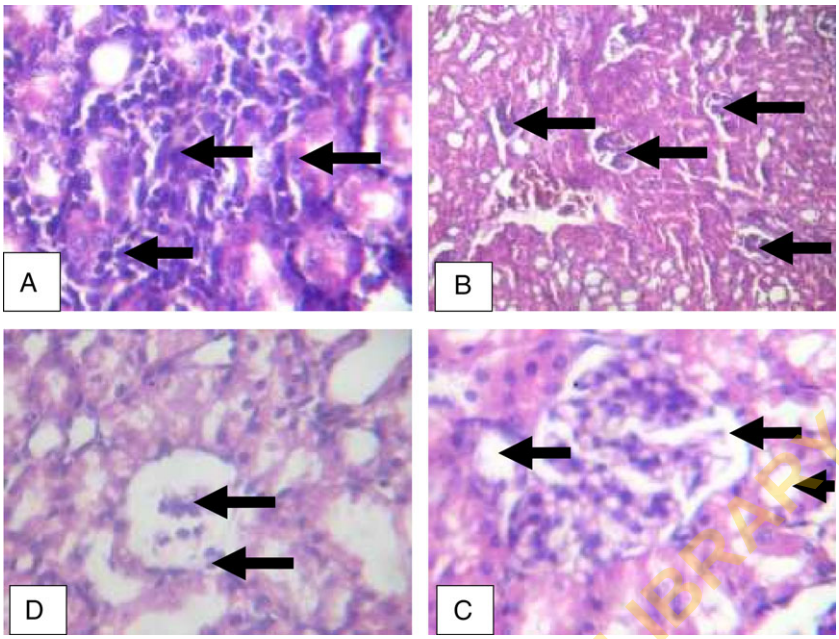


**FIG 2** *Photomicrograh showing kidney of rats (Group A) Control: show focal area of congestion of vessels with focal area of moderate interstitial infiltration by inflammatory cells (black arrows); (Group B) administered with (NaF 300 ppm alone) shows focal area of glomerular atrophy together with severe cellular infiltration of the renal tubules (black arrow); (Group C: NaF 300 ppm + quercetin 50 mg/kg) shows focal area of glomerular atrophy with mild cellular infiltration and (Group D: NaF 300 ppm + quercetin 50 mg/kg) show mild diffused hyaline and epithelial cell casts in the tubular lumen and fatty infiltration of the renal cortex (black arrow). Plates are stained with H and E stains and viewed with ×400 objectives.*



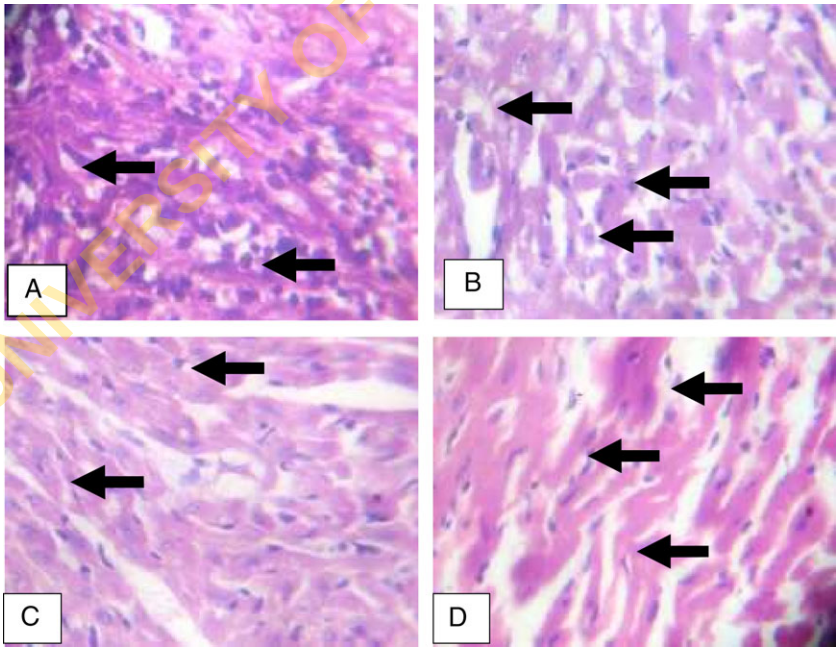


**FIG 3** *Photomicrograh showing heart of rats (Group A)-Control: shows diffused mild to moderate infiltration of the myocardium by inflammatory cells (black arrows); Group B: administered with (NaF 300 ppm alone) shows diffused mild infiltration of the pericardium and myocardium by inflammatory cells and focal area of fatty infiltration of the myocardium (black arrow); (Group C): NaF 300 ppm + quercetin 50 mg/kg) shows diffused very mild infiltration of the myocardium by inflammatory cells and focal area of fatty infiltration of the myocardium (black arrow), and (Group D) administered NaF 300 ppm + quercetin 50 mg/kg; shows diffused mild infiltration of the myocardium by inflammatory cells (black arrow). Plates are stained with H and E stains and viewed with ×400 objectives.*

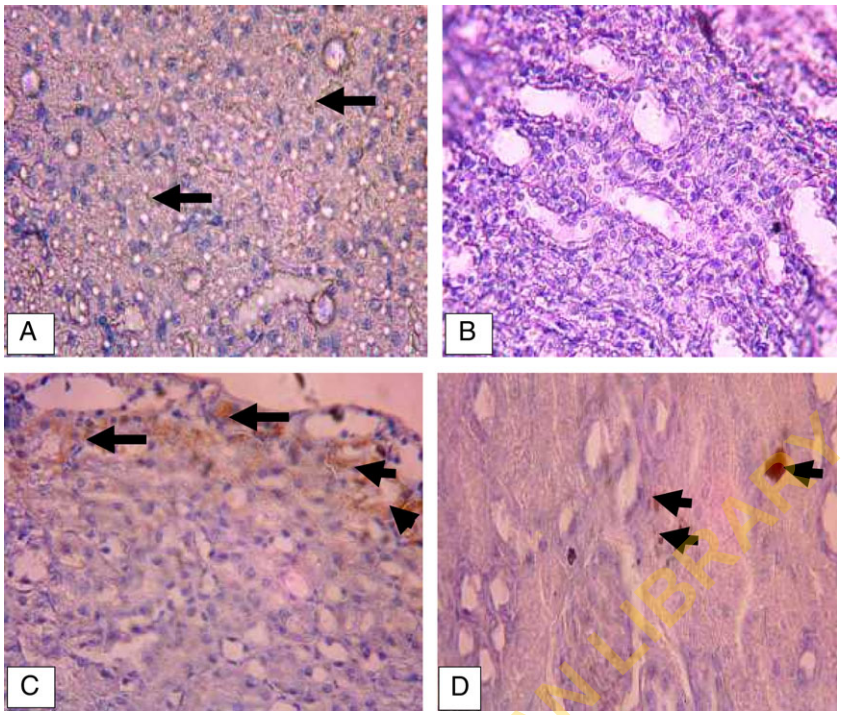


**FIG 4** *Immunohistochemistry of renal shock protein 70 (HSP70) in the kidney of rats. Group A: control: There is lower immune-positive reaction or HSP70. Group B (300 ppm NaF) shows no visible immune-positive reaction of HSP70. (Group C); NaF 300 ppm + quercetin 50 mg/kg) shows higher expression s or HSP70 when compared with the control and NaF only treated group (black arrow), and (Group D) administered NaF 300 ppm + quercetin 50 mg/kg; also shows higher expressions of HSP70 when compared with the control and NaF only treated group (black arrow). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives.*



### 4.2. Cardiac and renal markers of oxidative stress and antioxidant defense system

Reduced glutathione activity in cardiac tissues significantly fell in rats administered NaF alone when compared to Control. Also, protein thiol (PT) level in group co-administered with quercetin (100 mg/kg) decreased significantly in comparison with Control and rats administered NaF alone (Table 4). The renal level of GSH, non-protein thiol (NPT) and (PT) levels were unaffected by quercetin co-administration (Table 5). Furthermore, there were significant decreases in the activities of cardiac and renal SOD, CAT, GPx, and GST in rats administered NaF while the activities of SOD, CAT, GST, and GPx increased significantly in the cardiac and renal tissues of rats co-administered with quercetin (Tables 6 and 7). There was a significant increase in the cardiac markers of oxidative stress MDA and advanced oxidative protein Product (AOPP) in rats administered NaF alone compared with the Control and the increase in markers of oxidative stress was lowered in rats treated with quercetin (Table 8). In renal tissues, Hydrogen peroxide ($H_2O_2$) level increased significantly in rats administered NaF alone when compared with Control and quercetin treatment significantly attenuated the increase in the markers of oxidative stress (Table 9).

### 4.3. Electrocardiogram (ECG)

NaF significantly increased the heart rate, QRS, and QT interval when compared with control and that only the heart rate and the QRS values were ameliorated by quercetin co-treatment (Table 10).

### 4.4. Genotoxicity

The administration of NaF caused a significant increase in the frequency of MnPCEs as indicated in Fig. 1. However, co-administration of NaF with quercetin significantly lowered the frequency of MnPCEs (Fig. 1). This shows that quercetin has anti-genotoxic effect.

### 4.5. Histopathology

Histopathologic lesions of focal area of glomerular atrophy, severe cellular infiltration of the renal tubules, diffused infiltration of the pericardium and myocardium by inflammatory cells, and focal area of fatty infiltration of the myocardium were seen in rats exposed to NaF alone. However, these lesions were mild in groups treated with quercetin (Figs. 2 and 3).

### 4.6. Immunohistochemistry

NaF alone caused lower expressions of renal Hsp70 (Fig. 4), higher expressions of cardiac immune-positive expressions of Cyt C (Fig. 5), lower expressions of ERK in the aorta (Fig. 6),

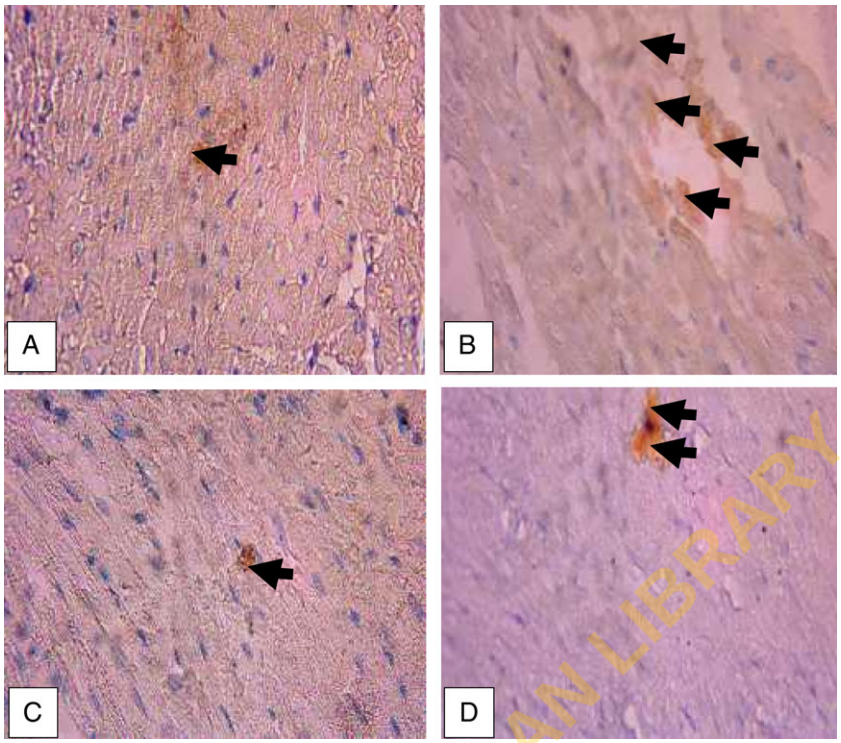


**FIG 5**

*Immunohistochemistry of cytochrome C in heart of rats. A: Control: There is lower immune-positive expression of cytochrome C. Group B (300 ppm NaF) shows higher immune-positive expression of cytochrome C when compared with the control. (Group C); NaF 300 ppm + quercetin 50 mg/kg) shows lower expressions of cytochrome C when compared with the NaF only-treated group (black arrow), and (Group D) administered NaF 300 ppm + quercetin 50 mg/kg; also shows lower expressions of cytochrome C when compared with the NaF only-treated group (black arrow). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives.*



higher expressions of renal p38 (Fig. 7), lower cardiac expressions of Hsp70 (Fig. 8), and expressions of lower PPAR-gamma of aorta (Fig. 9). On the other hand, co-administration of NaF with quercetin up-regulated the expressions of Hsp70, ERK PPAR-gamma along with the down-regulation of Cyt C and p38, respectively. Together, NaF enhanced cell death whereas treatment with quercetin promoted cell survival and abrogated cell death.

## 5. Discussion

Oxidative stress has been implicated in the pathogenesis of many diseases including hypertension with decreased antioxidant activity and increased production of ROS recorded in hypertensive patients [35]. In this study, the administration of NaF alone caused significant decrease in the activities of the antioxidant defense enzymes. Decreased activity of antioxidant enzymes such as CAT and SOD and reduced levels of ROS scavengers such as GSH have been reported to contribute oxidative stress [36] and may be indicative of excessive production of free radicals due to NaF exposure. In contrast, the aforementioned antioxidant enzymes were not significantly altered in rats treated with quercetin. Quercetin is a potent scavenger of reactive oxygen and nitrogen species [37], this may have accounted for the amelioration of the NaF-induced decrease in the systemic antioxidants observed in this study.

Also, the level of MDA and advanced oxidative protein Product (AOPP), increased significantly in rats administered NaF compared with rats treated with quercetin; strongly suggesting an aggravation of free radical production in excess of the amounts that can be processed by the endogenous antioxidant system, leading to increased lipid peroxidation and production of MDA as an advanced oxidation product [38,39]. Increased AOPP levels are often associated with increased carotid intima media thickness and may aid the development of arteriosclerosis [40,41]. The ability of quercetin to significantly reduce MDA, AOPP, and $H_2O_2$ levels in the kidney is indicative of the antioxidant property of quercetin and that might also contribute to the antihypertensive effect of quercetin as oxidative stress is involved in the pathogenesis of hypertension. The decreased diameter of the blood vessels and reduced vascular compliance invariably leads to increased blood pressure due to increased peripheral resistance. This may account for the significant increase in the blood pressure parameters, increase heart rate, QRS duration, and QT interval of rats administered

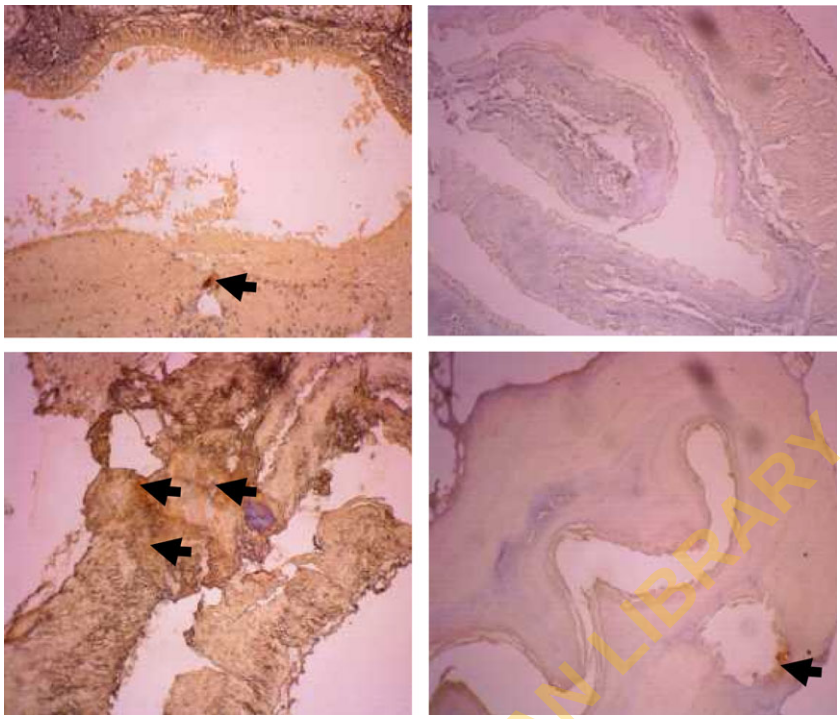

**FIG 6**

*Immunohistochemistry of extracellular signal regulated kinase (ERK) in aorta of rats. A –Control: There are high immune-positive expressions of ERK. Group B (300 ppm NaF) shows lower immune-positive expression of ERK when compared to the control. (Group C); NaF 300 ppm + quercetin 50 mg/kg) shows higher expressions of ERK when compared with the NaF only treated group (black arrow) and (Group D) administered NaF 300 ppm + Quercetin 50 mg/kg; also shows higher expressions of ERK when compared with the NaF only treated group (black arrow). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives.*



NaF alone. In this study, quercetin was able to ameliorate high blood pressure and restore the altered electrocardiographic changes. Under experimental conditions, SOD mimetics have been reported to ameliorate hypertension, probably by mitigating the effects of free radicals on the vasodilatory mechanisms of blood vessels [41–43]. This might result from the ability of SOD to quench superoxide anion radical thereby preventing the formation of peroxynitrite a damaging molecule to the vascular endothelium. Again, quercetin co-treatment with NaF caused significant improvement in the activities of renal SOD, CAT, and GST while cardiac CAT, GST, and GPx activities were also modulated. These together are indicative of antioxidant activity of quercetin. Micronucleated polychromatic erythrocytes has been reported as an important biomarker of DNA damage [44]. In this study, the observed increase in the frequency of MNPCE, showed the ability of NaF alter genomic integrity which is indicative of DNA damage [45]. However, co-treatment with quercetin significantly lowered the frequency of MNPCE. Increase in ROS generation by NaF might also contributed to increase in the frequency of MNPCE.

In addition to its potent antioxidant effect, quercetin reportedly inhibits inflammation because fewer ROS are available to activate the transcription factors NF-kB and AP-1 that induce proinflammatory cytokines [46]. By reducing inflammation, it is probable that quercetin upregulates NO synthase activity and enhance vasodilation in the blood vessels of rats, because higher amounts of NO are produced, and NO is a potent endogenous vasodilator. Observations in this study are similar to the reports of Roslan *et al*. [47], who reported the ameliorative effect of quercetin on oxidative stress-induced inflammation and alterations in blood pressure parameters in streptozotocin-nicotinamide-induced diabetes mellitus. Furthermore, quercetin has been reported to exert potent cardioprotective effect in oxidative stress mediated cardiomyocyte toxicity, and the inhibition of the progression of experimental autoimmune myocarditis via suppression of oxidative and endoplasmic reticulum stress through endothelin-1/MAPK signaling has been reported [48,49].

Heat shock proteins (HSPs) are highly conserved cellular stress proteins in every organism from bacteria to mammalian animals. Research reports have documented the importance of HSPs for cell survival under stressful conditions [50,51]. HSP70 acts as a molecular chaperon that responds to a wide variety of stress, such as heat shock, ischemia, and inflammation [52]. From this study, NaF reduced the expressions of Hsp70 in the heart and kidney of rats administered NaF alone whereas treatment with quercetin induced higher expressions of Hsp70. The experimental results may therefore suggest an antioxidant,

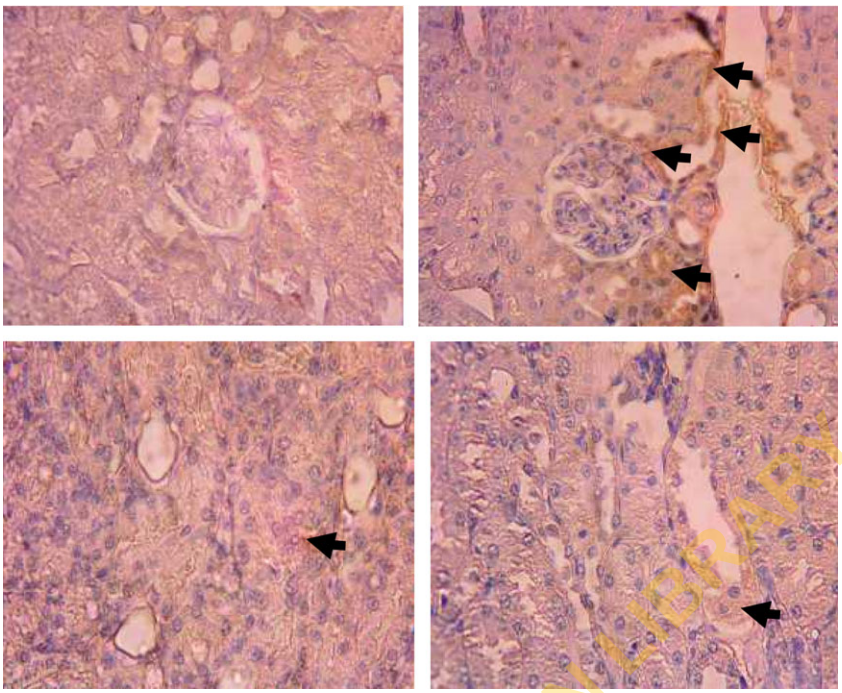

**FIG 7**

*Immunohistochemistry of renal p38 of rats. Group A -Control: There are high immune-positive expressions of p38. Group B (300 ppm NaF) shows lower immune-positive expression of p38 when compared with the control. (Group C); NaF 300 ppm + quercetin 50 mg/kg) shows higher expressions of p38 when compared with the NaF only-treated group (black arrow) and (Group DJ administered NaF 300 ppm + quercetin 50mg/kg; also shows higher expressions of p38 when compared with the NaF only-treated group (black arrow). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives.*



anti-inflammatory, and anti-apoptotic property of quercetin via up-regulation of Hsp70 as earlier reported [53,54]. From the same result, it can be proposed that quercetin has strong capacity to mitigate oxidative stress and inflammation through inhibition of translocation of NF-κB from the cytoplasm to the nucleus as reported earlier by Dokladny et al. [41] with the ultimate attenuation of myocardial and renal cell death.

Furthermore, the results of our study showed that administration of NaF alone was accompanied with higher expressions of Cyt C when compared with the rats treated with quercetin. This might also indicate that quercetin is a potent inhibitor of apoptosis and a promoter of cell survival. The release of Cyt C from the mitochondria has been associated with mitochondrial dysfunction, oxidative stress and apoptosis [55]. The mitochondrial **ROS** generation has been shown to contribute to the release of Cyt C and proapoptotic proteins from mitochondrial intermembrane space into the cytosol [56]. Hence, Cyt C release from mitochondria is considered a key step in the initiation of apoptosis in both intrinsic and extrinsic pathways [57,58]. Therefore, preventing Cyt C release from the mitochondria may be a therapeutic target against apoptosis [59].

It has been reported that cardiac cell death and fatal arrhythmias during myocardial ischemia/reperfusion (I/R) can be reduced by p38 MAPK inhibition [60]. Our data showed that NaF reduced the expressions of aortic ERK. On the other hand, co-administration of quercetin with NaF increased the expressions of ERK in the aorta. The MAPKs are ubiquitous intracellular signals, consisting of extracellular signal regulated kinase 1/2 (ERK1/2), p38, and c-Jun NH2-terminal kinase (JNK) [61]. The MAPK family of serine/threonine-specific protein kinases is known to mediate cellular adaption, growth, survival, apoptosis, proliferation, differentiation, metabolism, and motility [62,63]. From the present study, NaF increased the expressions of p38 in the renal tissues of rats. This might be an indication of oxidative stress and renal cell death following the administration of NaF. Previous studies have shown that NaF-induced apoptosis via upregulation of p38 and downregulations of both p-ERK and total ERK [64,65]. Therefore, quercetin administration was able to quench oxidative and ameliorate apoptosis as observed in the lower expressions of p38.

Also, in this study, administration of NaF only reduced the expression of peroxisome proliferator-activated receptor-gamma (PPARγ) in the aorta of rats while pre-treatment with quercetin increased the expressions of (PPARγ). Increased expressions of the PPARγ might be indicative of cardioprotective effect of quercetin on NaF-induced cardiovascular dysfunction. Recent studies have reported the cardioprotective effect of PPARγ [66,67]. Hence, quercetin may serve as another potent PPARγ agonist. The involvement of PPARγ in cardiovascular lipid homeostasis and diabetes has been reported [68]. The antioxidant, anti-inflammatory, and cardioprotective effects of PPARγ against I/R injury has been reported [69,70].

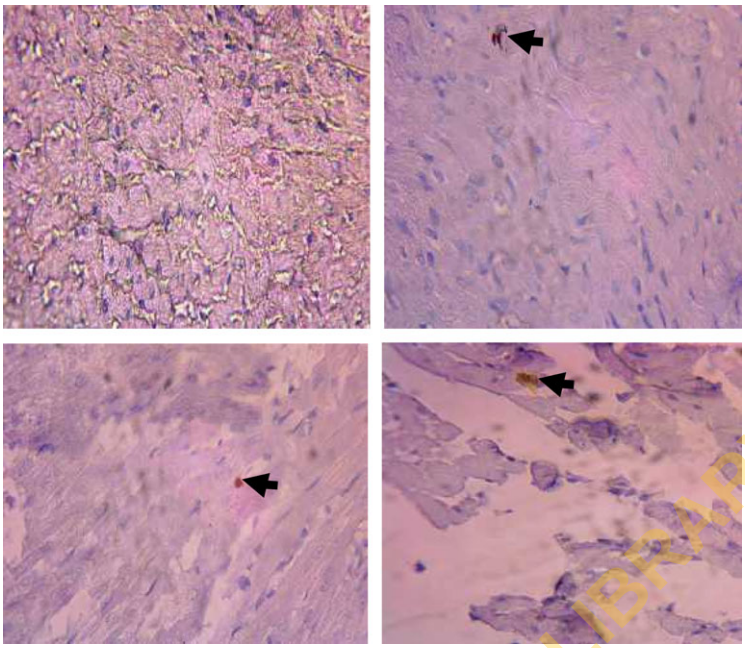

**FIG 8** *Immunohistochemistry of cardiac HSP70 of rats. Group A-Control: There are high immune-positive expressions of HSP70. Group B (300 ppm NaF) shows lower immune-positive expression of HSP70 when compared with the control. (Group C); NaF 300 ppm + quercetin 50 mg/kg) shows higher expressions of HSP70 when compared with the NaF only treated group (black arrow), and (Group D) administered NaF 300 ppm + quercetin 50 mg/kg; also shows higher expressions of HSP70 when compared with the NaF only-treated group (black arrow). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives*

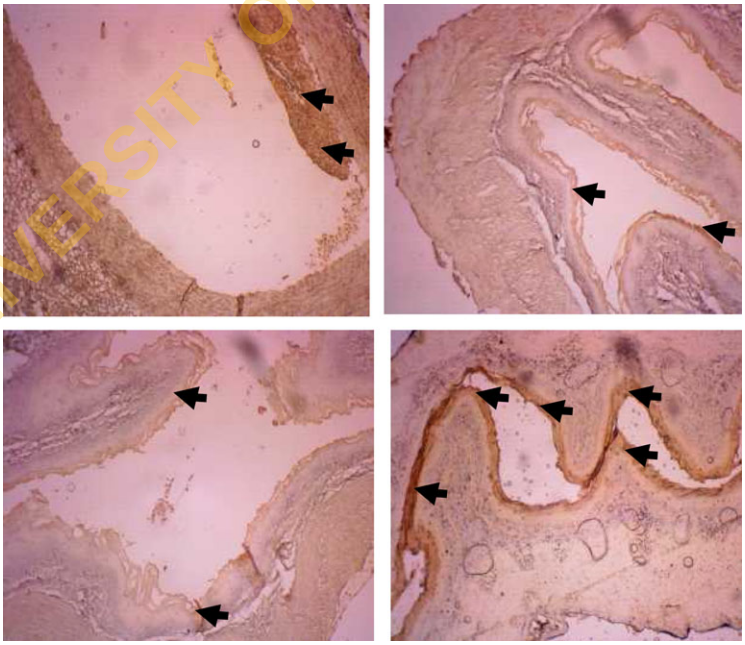

**FIG 9** *Immunohistochemistry of PPAR-gamma of aorta of rats. Group A-Control: There are high immune-positive expressions of PPAR-gamma. Group B (300 ppm NaF) shows lower immune-positive expression of PPAR-gamma when compared to the control. (Group C); NaF 300 ppm + quercetin 50 mg/kg) shows higher expressions of PPAR-gamma when compared with the NaF only treated group (black arrow), and (Group D) administered NaF 300 ppm + quercetin 50 mg/kg; also shows higher expressions of PPAR-gamma when compared with the NaF only treated group (black arrow). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives.*

## 6. Conclusion

NaF administration increased blood pressure parameters accompanied by decrease in the activities of cardiac and renal antioxidant enzymes, as well as increases in the markers of oxidative stress and DNA damage. Co-administration of NaF along with quercetin attenuated the toxic effects of NaF.

From this study, quercetin was able to quench NaF-induced free radical generation, increased the expressions of ERK, HsP70, and PPARγ which is indicative of its antioxidant, anti-inflammatory, antiapoptotic, and cardioprotective effects. Similarly, remarkable reductions of p38 and Cyt C was observed in rats treated with quercetin. Therefore, it may be proposed that co-administration of quercetin with NaF lessened oxidative stress, improved endothelial function and reduced hypertension. Together, fruits and vegetables that are rich in quercetin may be considered as a potential candidate for the treatment of hypertension, cardiovascular dysfunctions, and its associated complications.

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## Conflict of interest

The authors declare no conflict of interest.

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