Immunopathologia Persa
DOI:10.34172/ipp.2025.43763
http immunopathol.com

Effect of garlic on sodium fluoride-induced hypertension and elevated cardiac enzymes in male albino rats

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Received 6 Oct. 2024
Revised: 4 Jan. 2025
Accepted 22 Jan. 2025
ePublished 8 Mar. 2025

Keywords: Sodium fluoride, Garlic, *Allium sativum*, Oxidative stress parameters, Lipid profile

Citation: Badr El-Din S, ALfiky HAA, Wahsh E, Badawy H, Seliem N, El Attar Sh, Mehanna MG, Farahat AMA, Ibrahim MM, Hafiz WFA, Ahmed MI, El Nady M, Hewady PM, Ahmed SA, Badr B, Abd-Elrhman A, Hassan DAA. Effect of garlic on sodium fluoride-induced hypertension and elevated cardiac enzymes in male albino rats. Immunopathol Persa. 2025;11(2):e43763. DOI:10.34172/ipp.2025.43763.

Abstract

Introduction: Garlic, also known as *Allium sativum*, has been used for centuries to protect the heart and reduce blood pressure.
Objectives: This study aims to evaluate the antihypertensive effects and cardioprotective effects of garlic against the effects of sodium fluoride (NaF) on the blood pressure and heart tissue.
Materials and Methods: Thirty adult male Albino rats used in the study. Rats were assigned into three groups consisting of one control group, NaF, and NaF + garlic. After a week of acclimatization, the rats were sedated with ketamine and thoracotomy, blood samples were collected, and heart tissues were dissected. Heart specimens were taken from the left ventricle and stained with hematoxylin and eosin for histological examination. Masson's trichrome was conducted to examine collagen fibers.
Results: The study findings indicated that exposure to NaF leads to an increase in indicators of cellular demise and a decrease in markers of survival in cardiac tissue, suggesting the occurrence of apoptosis. This exposure also disrupts blood lipid profiles, resulting in elevated cholesterol and triglyceride levels and reduced high-density lipid (HDL) levels. Furthermore, it enhances the activity of oxidative stress enzymes while lowering the levels of glutathione (GSH). Conversely, garlic exposure is associated with decreased arterial blood pressure, improved histopathological structure, and reduced serum levels of oxidative stress biomarkers.
Conclusion: After thorough evaluation, it has been determined that garlic therapy may hold potential as a treatment for various cardiovascular diseases.

Introduction

One environmental pollutant that has been overlooked and poses a threat to both human and animal health is sodium fluoride (NaF) (1). NaF can be found in soil, water, and the atmosphere, and has been linked to a range of cardiovascular and renal system dysfunctions due to its ability to induce oxidative stress (2). The existence of fluoride in bodily tissues was accompanying by structural alterations

Key point

In this research study, 30 adult male albino rats were categorized into three groups: the control, sodium fluoride (NaF), and NaF + GA groups. Following the respective treatments, the rats' arterial blood pressure was recorded, their lipid profiles and oxidative stress enzyme levels were assessed, and their heart tissues were dissected and subject to histological examination. This study findings suggest that exposure to NaF is associated with elevated arterial blood pressure, heightened markers of cellular death, and diminished indicators of survival in cardiac tissue. NaF is also linked to disruptions in blood lipid profiles and increased levels of free radicals. Conversely, garlic demonstrates the ability to enhance antioxidant enzyme activity, reduce blood pressure, and restore normal cardiac tissue function.

and functional disturbances (3). In severe instances, acute fluoride poisoning can result in rapid cardiac fatality, believed to stem from a marked decrease in calcium levels due to the formation of calcium fluoride salt. Chronic fluorosis, stemming from prolonged increased fluoride intake, is prevalent in various global regions, posing a threat to both human and animal health. Given the established link between fluoride exposure and the stimulation of reactive oxygen species (ROS) overproduction, which compromising the body's antioxidant defense system, using products with antioxidant properties might help reduce the harmful effects of fluoride (4).

Hypertension is a prevalent disorder and serves as a significant risk factor for cardiovascular disease and mortality. For instance, changes in the left ventricle caused by hypertension may result in coronary artery diseases, and eventual fatality. An estimated 1.4 billion individuals globally have been affected by hypertension and its associated complications. The effective management and regulation of hypertension are paramount in preventing cardiovascular diseases and their related conditions (5). Recent guidelines advocate the utilization of alternative natural products in the management of hypertension (6). Certain studies have directed their focus toward the use of natural compounds in regulating gut microbial flora to control hypertension (7).

Garlic, or *Allium sativum*, has been historically utilized to promote cardiovascular health and reduce blood pressure (8). Nevertheless, meta-analysis investigating its impact on hypertension have yielded inconclusive findings.

Recent studies indicate that garlic supplements may effectively reduce blood pressure in individuals with hypertension (9). Research involving Kwai garlic powder has demonstrated that doses between 600 and 900 mg daily can significantly impact hypertension without affecting normal blood pressure (5). Furthermore, incorporating a meal supplemented with garlic homogenate has been found to lower systolic and diastolic blood pressure in patients with hypertension (10). Additionally, aged garlic extract, a type of garlic preparation aged for over ten months, has shown promise in effectively lowering blood pressure in individuals with uncontrolled hypertension. Aged garlic extract has also exhibited the ability to improve blood flow in hypertensive rats, increase nitric oxide (NO) concentrations in mice, and induce relaxation of isolated rat aortic rings (5).

Objectives

The current study is focused on evaluating the effects of garlic extract on arterial blood pressure, cardiac tissue histology, and biochemical parameters in male albino rats induced with hypertension via NaF.

Materials and Methods

This research was carried out in the Department of Pharmacology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. Thirty mature albino male rats, weighing between 180 g and 220 g, were acquired from the farm of the "National Organization for Drug Control and Research" in Giza, Egypt. The animals were given one week to adapt to their environment. The rats were kept in a precisely controlled environment with a 12-hour light/dark cycle. The rats were kept at a consistent temperature of 23-27 °C and a relative humidity of 52%–58%, with continuous access to their standard pellet meal. The rats were acclimatized to their feeding regimen one week prior to the experiment.

Study protocol

After one week of acclimatization, these animals were allocated randomly into three groups (n=10) as follows:

- Group I (CN): Control group, where animals received no treatment.
- Group II (NaF): The animals received oral NaF at 10 mg/kg BWT daily.
- Group III (NaF + Ga): The animals were given NaF orally at 10 mg/kg BWT/day and garlic at 63 mg/kg BWT/day (11).

Sacrifice of rats and specimen collection

Following the conclusion of the treatment (four weeks), rats had a 24-hour fasting period overnight. Thereafter, rats from each group were anesthetized with ketamine (60 mg/kg IP, intraperitoneal), underwent thoracotomy, blood samples were collected, and heart tissues were immediately removed and stored in 10% formol saline for one day.

Arterial blood pressure

Rats were fasted overnight for 6–8 hours and sedated with urethane (1200 mg/kg IP). After examining the animal's reflexes, it was placed on a flat, movable surface. After administering anesthesia, the skin on the ventral side of the neck is cleaned and shaved. A little incision measuring 1.5-2 cm was executed in the neck to cannulate the carotid artery and facilitate a tracheostomy. Sterile polyethylene (PE) tubing and a 26 G×1/2½ needle pre-filled with heparinized normal saline (0.5 IU/mL) were utilized for blood vessel cannulation. The cannulated blood vessel was subsequently

linked to a pressure transducer, Power Lab 4/35 hardware, and Power Lab Chart Pro software (AD Instruments, Australia) for the measurement of blood pressure.

Blood samples were collected from the rats. Enzymatic assays assessed the lipid profile, including total cholesterol (TC), triglycerides (TG), high-density lipid (HDL), and low-density lipid (LDL). Cardiac enzymes, including creatine kinase (CK), creatine kinase-MB (CK-MB), cardiac troponin I (cTNI), and LDH were evaluated using the enzyme-linked immunosorbent assay (ELISA) technique.

Biochemical oxidative parameters

The heart was excised, rinsed in ice-cold 0.175 M KCl / 25 mM Tris–HCl (pH 7.4) to remove the blood, minced in the same solution, and homogenized by means of a homogenizer with a Teflon pestle. The heart homogenates were centrifuged at 10 000 rpm for 15 minutes. The supernatants were then used for lipid peroxidation determination and antioxidant enzyme assays as follows:

a. Tissue glutathione (GSH) analysis: The reduced GSH content of thyroid tissue was estimated according to the method described by Sedlak and Lindsay (12).
b. Tissue superoxide dismutase (SOD) and catalase (CAT) activity determination: The SOD activity was measured by the inhibition of nitro blue tetrazolium (NBT) reduction due to O_2 generated by the xanthine/xanthine oxidase system (13). One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The CAT activity of tissue was determined according to the method of Sinha (14). The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was conducted as a measure of CAT activity. The enzyme activity was given in U/mg of protein.
c. Determination of malondialdehyde (MDA) levels: The levels of MDA in homogenized tissue, as an index of lipid peroxidation, were determined by a thiobarbituric acid reaction using the method of Yagi (15).
d. Determination of protein content: The tissue protein content was measured using bovine serum albumin (BSA) as a standard (16).

Histological methods

Formalin-fixed heart specimens were taken from the left ventricle for light microscopy. Tissue sections were cut (typically 4-5 µm thick) from the paraffin-embedded blocks using a microtome, mounted, and baked to the glass slides, and finally dewaxed using xylene and rehydrated by graded alcohols to be ready for staining.

Hematoxylin and eosin: for routine histological examination, sections were stained with hematoxylin and eosin for visualizing tissue structure and morphology.

Masson's trichrome: sections were stained with Masson's trichrome to examine collagen fibers.

Immunohistochemistry

Inflammatory marker (tumor necrosis factor-alpha, TNF-α)

Sections obtained were immersed in citric acid buffer solution and heated three times in a microwave oven (3 minutes heating and 5 minutes braising each time) to realize sufficient antigen retrieval endogenous peroxidase blocker was added to be reacted for 10 minutes. The specimens were rinsed and added with goat serum for 20 minutes, and anti-TNF-α primary antibodies (1:200) were added after the goat serum blocking buffer was shaken off, followed by culture in a refrigerator at 4 °C overnight. Sections were rinsed and added with secondary antibody solution to be reacted for 10 minutes. After rinsing adequately, the streptavidin-per-oxidase solution was added for a reaction for 10 minutes, followed by color development with 3,3-diaminobenzidine (DAB) (Solarbio, Beijing, China) in drops. Tissue sections were counterstained for the nucleus by hematoxylin to visualize cellular morphology.

Apoptotic (Bax) and antiapoptotic (Bcl-2) markers

The slides were incubated for 2 hours with 5% BSA in tris-buffered saline (TBS), followed by overnight immunostaining at 4 °C with primary antibodies Bax (catalog number PA5-11378) and Bcl-2 (catalog number PA5-20068) at a concentration of 1 µg/mL in 5% BSA in TBS. Subsequent to washing the slides with TBS, they were treated with the appropriate goat anti-rabbit secondary antibody. After washing with TBS, incubation was conducted for 10 minutes in a solution of 0.02% diaminobenzidine containing 0.01% hydrogen peroxide. Counterstaining was performed utilizing hematoxylin.

Morphometric studies

The Olympus® CX41light microscope (×400) was employed to investigate and capture images of 10 non-overlapping fields from each slide, utilizing an "Olympus® SC100" digital camera. The morphometric investigation was conducted in accordance with the program instructions, utilizing the ImageJ image analysis software (NIH, USA) program. The area percentage of collagen fibers in Masson's trichrome-stained sections was determined in 10 non-overlapping fields per slide. The same program was also employed to quantify TNF-α, Bcl-2, and Bax immunostaining. The results were displayed as the optical density of a positive TNF-α expression per area and the "optical density" of all Bcl-2 and Bax stained sections per area.

Statistical analysis

Data was gathered utilizing the SPSS version 22 software and presented as the mean ± SD. Group comparisons were conducted using one way analysis of variance (ANOVA)

Table 1. Arterial blood pressure (systolic, diastolic, and mean arterial blood pressure) in control (GI), NaF (sodium fluoride) (G2), and NaF + G treated (G3)

Blood pressure (mm Hg)	Experimental groups			F-test	*P* value
	Control (G1)	NaF (G2)	NaF + Ga (G3)		
Systole (mm Hg)	95.01±7.14 c	154.02±1.52 a	100.04±1.22 b	61.127	<0.001**
Diastolic (mm Hg)	66.30±6.11 c	110.10±8.00 a	70.10±6.00 b	45.204	<0.001**
Mean arterial blood pressure (mm Hg)	90.12±8.16 c	150.01±5.11 a	94.01±3.14 b	71.134	<0.001**

Data are expressed Mean ± SD: mean and standard deviation values in each row with different letters are significantly different at (P<0.05).
** P value <0.001 is considered highly significant.
'a' represents the highest blood pressure measurement among the study groups.
'b' indicates an intermediate blood pressure reading, elevated compared to the control group yet diminished relative to the NaF group.
'c' represents the minimum blood pressure reading among the three groups. It is linked to the control group.

test and student's t-test to compare between two groups. Statistical significance was observed at $P \leq 0.05$..

Results

Table 1 illustrates a statistically significant increase in systolic, diastolic, and mean arterial blood pressure in the NaF group ($P < 0.001$) compared to the control group. Notably, the administration of garlic led to a significant reduction in blood pressure, encompassing systolic, diastolic, and mean arterial blood pressure, as depicted in Figure 1 and Table 1.

Histological, immunohistochemical and statistical results

Hematoxylin and eosin-stained sections results

Cardiac muscle fibers of the control group showed regular arrangement with apparent normal striations and branching forming network. Scanty intercellular spaces containing small blood capillaries were also evident. Cardiac muscle fibers exhibited acidophilic sarcoplasm and centrally located oval nuclei. Histological analysis of the NaF group demonstrated significant deformation, fragmentation, and loss of striation in heart muscle. Cardiac muscle fibers exhibited hyper-eosinophilia in the cytoplasm alongside peripheral pyknotic nuclei. A significant increase in

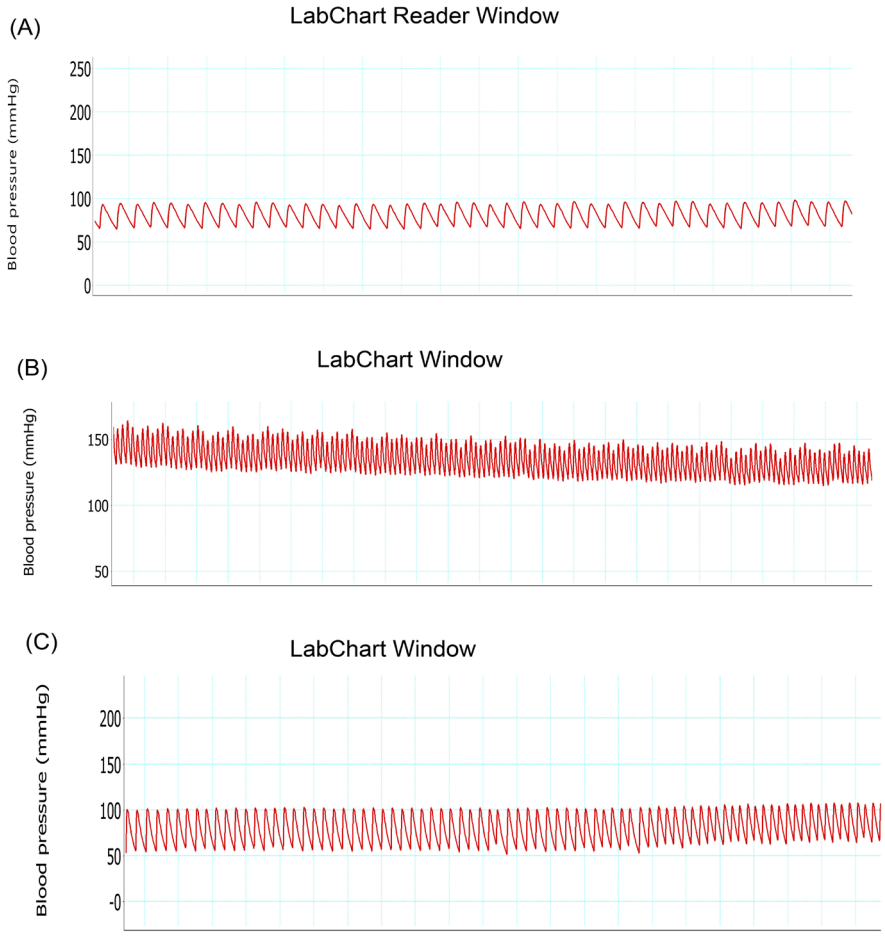

Figure 1. A rhythm strip showing blood pressure changes in (A) control group, (B) sodium fluoride group, (C) NaF+Ga group.

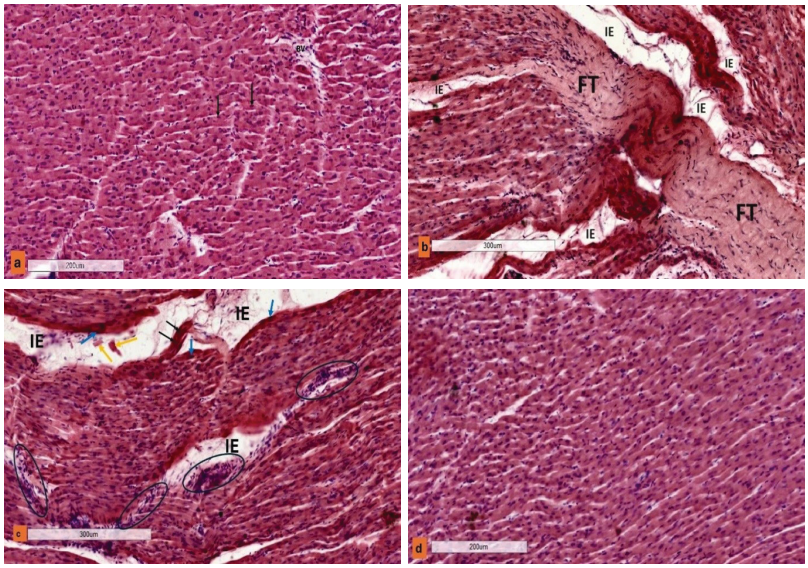

Figure 2. H&E-stained representative photomicrographs of heart sections. **(a)** control rat demonstrating the regular arrangement of the myocardial bundles, scanty tissue spaces, blood capillaries (BV), acidophilic cytoplasm with apparent striations and central vesicular nuclei (arrows). **(b & c)** NaF-treated rat showing the irregular arrangement of the myocardium, a noticeable increase in the tissue space with "Interstitial Edema" (IE), inflammatory cell infiltrations, fibrous tissue deposition (FT) between muscle fibers, marked distortion, fragmentation (yellow arrows) of muscle fibers, hyper-eosinophilic cytoplasm (black arrows) with pyknotic and peripheral nuclei (blue arrows), between cardiac muscle fibers. **(d)** NaF+Ga co-treated rat showing the normal structure of the cardiac muscle.

tissue gaps with interstitial edema, inflammatory cellular infiltration, and fibrous tissue deposition among cardiac muscle fibers was noted. The NaF+Ga co-treated group exhibited significant preservation of cardiomyocyte shape, organization, and tissue spacing, resembling the control group closely. Myocardial cells showed acidophilic striation in the cytoplasm and central vesicular nuclei (Figure 2).

Masson's trichrome-stained sections results

The control group exhibited negligible blue-stained collagen fibers interspersed among the heart muscles. The NaF-treated group exhibited an augmentation in collagen fiber deposition, demonstrating a statistically significant increase in its percentage area relative to the control group. The NaF+Ga co-treated group showed few blue stained fibers with a statistically significant decrease in its percentage area compared with the NaF group (Figure 3).

Immunohist1ochemical results

Inflammatory markers

The effect of NaF on inflammatory cascades was assessed by measuring the cardiac expression of TNF-α. The tissue with positive TNF-α expression were sepia. There was lower positive expression in the control and NaF+Ga co-treated groups and higher positive expression in the NaF group. The statistical results showed that the average optical densities of TNF-α positive expression rose significantly in the NaF group compared to the control group. Compared with the NaF group, the NaF+Ga group had reduced the average density of the positive expression of TNF-α (Figure 4). Differences in the average optical densities of TNF-α positive expression between all studied groups were also shown in (Table 2).

Apoptotic and antiapoptotic markers

The apoptotic tissue damage caused by NaF was evaluated using immunohistochemical analysis of the "pro-apoptotic marker" (Bax) and the "anti-apoptotic marker" (Bcl-2) in the myocardium. The control group showed almost negative immunostaining for Bax and intense immunostaining for Bcl-2. Notably, NaF significantly elevated the expression of Bax and significantly reduced the expression of Bcl-2 compared to the control group, as evidenced by the intense heavy (brown staining) Bax expression and minimal Bcl-2 expression. On the other hand, NaF+Ga co-treated ameliorated these changes, as evidenced by mild Bax expression and extensive Bcl-2 expression, which proved to be of significant values if compared with the NaF group (Figure 5). Differences in the optical densities of Bax and Bcl-2 expression between all studied groups were also shown in (Table 2).

As Table 3 shows, in terms of total CK, it increased in the NaF group (mean ± SD: 741 ± 16) compared with that of the NaF+Ga group (341 ± 8 U/L) and that of the control group (125 ± 1.7 U/L), with high statistically significant ($P < 0.001$) differences (between all three groups, between NaF and control groups, between NaF and NaF +Ga groups). Regarding CK-MB, it was increased in the NaF group (458 ± 1.6 IU/L) when compared with that of the NaF+Ga group (301 ± 2.3 IU/L) and that of the control group (280 ± 1.7 IU/L), with high statistically significant ($P < 0.001$) differences (between all three groups, between NaF and CN (control) groups, between NaF and NaF+Ga groups.

Regarding cTN 1, it was increased in the NaF group

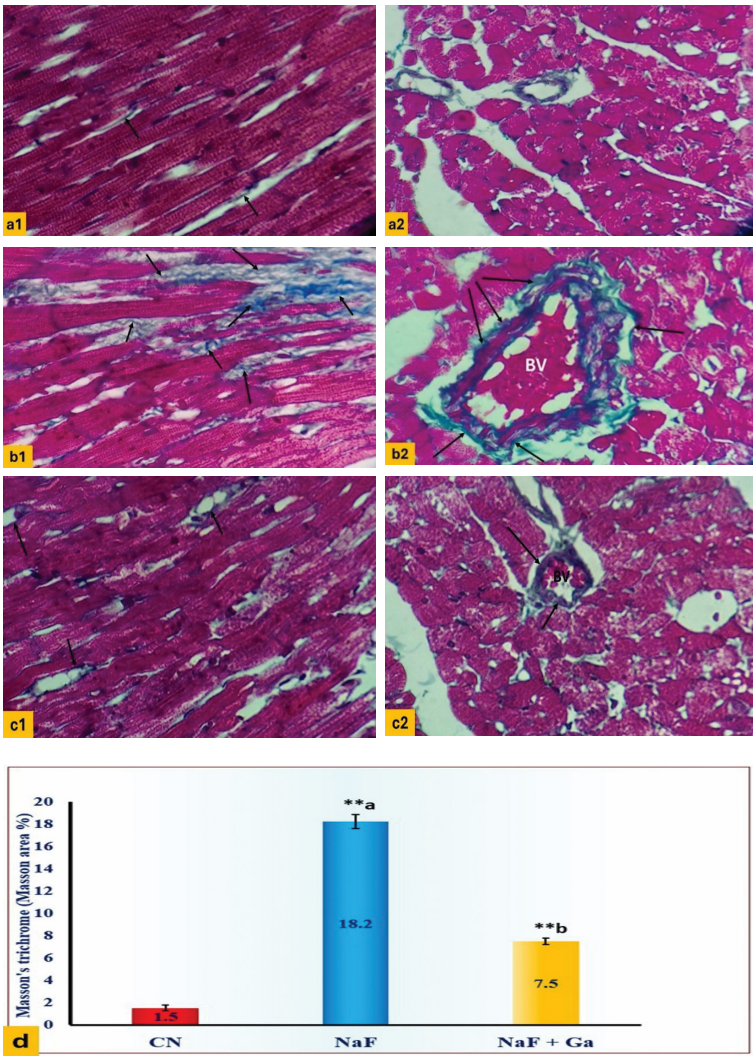

Figure 3. Microscopic figures and statistical analysis of heart sections stained by Masson's trichrome (Magnification×400). **a:** Control rats exhibit few blue-stained collagen fibers (arrows) interspersed among cardiac muscle fibers (**a1**) and surrounding blood vessels (BV) (**a2**). **b:** NaF-treated rats demonstrate dense blue-stained collagen fibers (arrows) interspersed throughout the heart muscle fibers (**b1**) and surrounding congested blood vessels (BV) (**b2**). **c:** NaF+Ga co-treated rat showing some blue-stained collagen fibers (arrows) between cardiac muscle fibers (**c1**) and around blood (BV) vessel (**c2**). **d:** Histogram showing the Masson's trichrome stained area percentage in different groups. Data are presented as mean ± SD (n =10). CN=Control group, NaF= Sodium fluoride-treated group, NaF+Ga=Sodium fluoride+ Garlic treated group, ***P*<0.001, [a] versus control group. [b] versus sodium fluoride group.

(0.76 ± 0.06 ng/mL) when compared with that of the Naf + Ga group (0.36 ± 0.17 ng/mL) and that of the control group (0.34 ± 0.03 ng/mL), with high statistically significant ($P < 0.001$) differences (between all three groups, between NaF and control groups, between NaF and NaF+Ga groups. In relation to LDH, it was increased in the NaF group (540 ± 3.1 U/L) when compared with that of the NaF+Ga group (398 ± 9.4 U/L) and that of the control group (360 ± 3.2 U/L), with high statistically significant ($P < 0.001$) differences (between all three groups, between

Table 2. Percentage of Masson's trichrome stained areas, optical density of a positive TNF-α expression per area, and optical density of each of Bcl-2 and Bax expression per area in different groups

	Experimental groups			F-test	T-test (a)	T-test(b)	*P* value (F-test/T-test a/T-test b)
	CN (G1)	NaF (G2)	NaF +Ga (G3)				
Masson's trichrome (Masson area %)	1.5±0.26	18.2±0.62	7.5±0.28	6080	-96.2	60.9	<0.001**
Immunohistochemistry TNF-α (OD/Area)	0.205±0.047	0.816±0.078	0.417±0.058	740	-36.7	22.5	<0.001**
Immunohistochemistry BAX (OD/ Area)	57±4.29	86.4±3.69	62.4±4.12	451	-28.5	23.8	<0.001**
Immunohistochemistry Bcl-2 (OD/ Area)	76.3±2.05	52.3±3.3	66±2.95	549	34	-16.9	<0.001**

** *P* value <0.001 is considered highly significant, T- test comparing means of each 2 groups
CN: Control group, NaF: Sodium fluoride group; NaF+Ga: Sodium fluoride+garlic.
The values in Table 2 were expressed as mean ± SD, where CN is the control group, NaF is the NaF orally treated group (10 mg/kg/d for 4 weeks), NaF+Ga is the group co-treated orally with NaF (10 mg/kg/d for 4 weeks) and Garlic (63 mg/kg/d for 4 weeks). (a) NaF group versus control group, (b) NaF+Ga group versus NaF group. Values in each row are significantly different at ($P<0.001$).

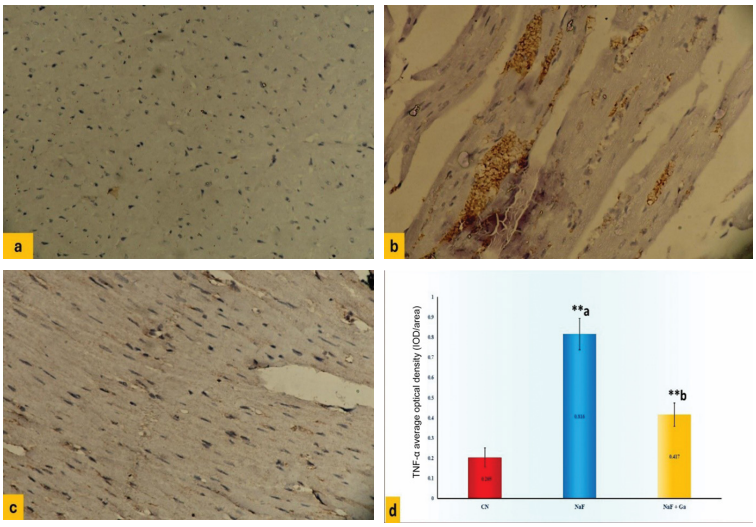

Figure 4. Immunohistochemical figures and statistical analysis of TNF-α in heart sections (Magnification×200). **a**: control rat showing minimal TNF-α expression. **b**: NaF-treated rat showing extensive heavy TNF-α expression. **c**: NaF + Ga co-treated rat showing lower positive TNF-α expression. **d**. Histogram showing quantitative image analysis for immunohistochemical staining of TNF-α respectively expressed as optical density/Area (IOD/area) across ten different fields for each rat section. Data are presented as mean ± SD (n =10), CN= Control group, NaF=Sodium fluoride-treated group, Naf + Ga = Sodium fluoride + Garlic treated group, **P<0.001, a versus control. b versus sodium fluoride.

NaF and control groups, between NaF and NaF+Ga groups (Table 3).

As Table 4 demonstrates, in relation to the TC, it exhibited an increase in the NaF group (135 ± 1.4 mg/dL) compared to the NaF+Ga group (90 ± 1.4 mg/dL) and the control group (75 ± 0.8 mg/dL). These differences were found to be statistically significant (P < 0.001) between all three groups, as well as between the NaF and control

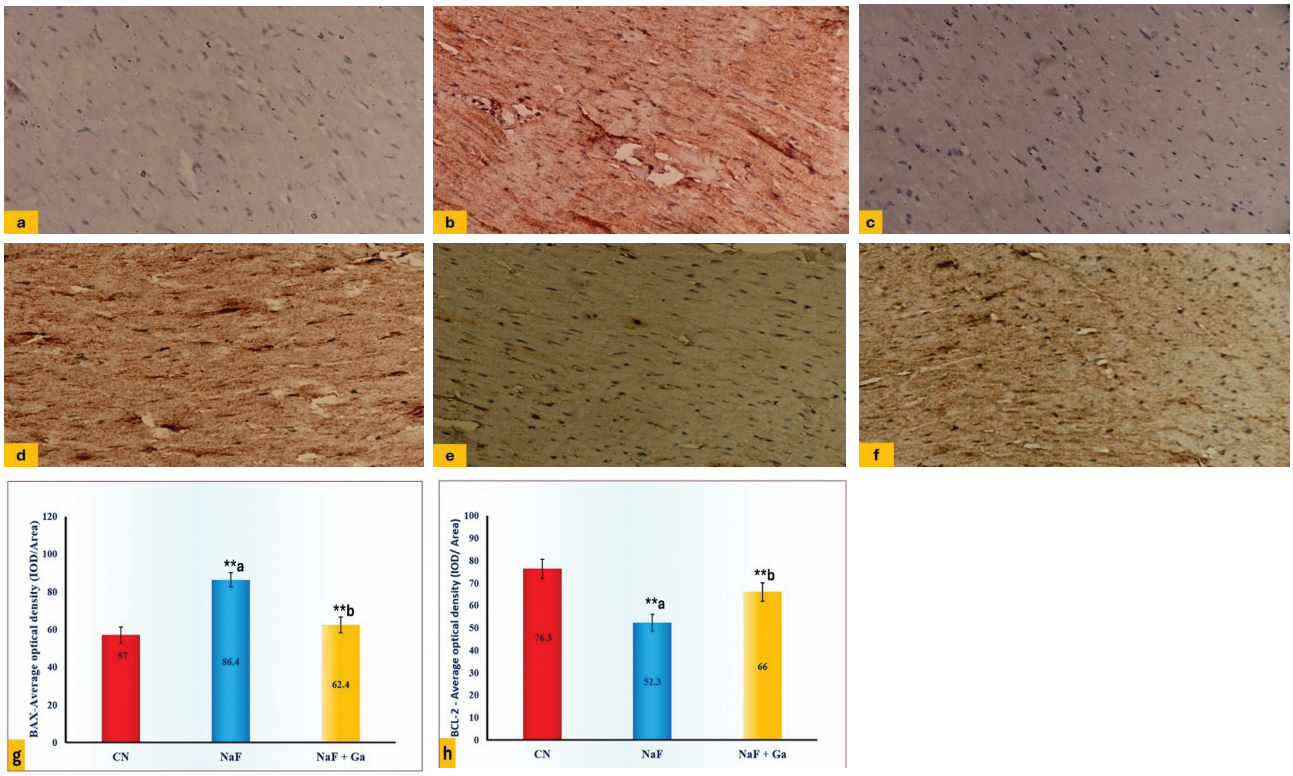

Figure 5. Immunohistochemical figures and statistical analysis of Bax and Bcl-2 in heart sections (Magnification×200). **a and d:** control rats showing minimal Bax and maximal Bcl-2 expression, respectively. **b and e:** NaF-treated rats showed extensive heavy Bax and minimal Bcl-2 expression, respectively. **c and f:** NaF+Ga co-treated rats showing mild Bax and extensive Bcl-2 expression, respectively. **g and h**: Histograms showing quantitative image analysis for immunohistochemical staining of Bax and Bcl-2, respectively, each expressed as optical density /area (IOD/area) across ten different fields for each rat section. Data are presented as mean ± SD (n = 10), CN = Control group, NaF = Sodium fluoride-treated group, NaF+Ga = Sodium fluoride+garlic treated group, **P<0.001, a versus control group. b versus sodium fluoride group.

groups, and between the NaF and NaF +Ga groups.

With respect to TG, the levels were observed to be higher in the NaF group (105 ± 1.7) when compared to both the NaF + Ga group (Mean ± SD: 91 ± 2 mg/dL) and the control group (Mean ± SD: 76.7 ± 1.7 mg/dL). These differences were found to be statistically significant ($P < 0.001$) across all three groups, as well as specifically between the NaF and control groups and the NaF and NaF+Ga groups. In relation to HDL levels, a reduction was observed in the NaF group (.6 ± 2.1 mg/dL) compared to both the NaF+Ga group (38.3 ± 2.1 mg/dL) and the control group (43.3 ± 1.2 mg/dL). These differences were found to be statistically significant ($P < 0.001$) between all three groups, as well as between the NaF and control groups, and the NaF and NaF +Ga groups. In terms of LDL, the NaF group exhibited higher levels (36.8 ± 2.4) compared to both the NaF + Ga group (24.9 ± 1.6 mg/dL) and the control group (35 ± 11.2 mg/dL). This difference was found to be statistically significant ($P1 = 0.001$) across all three groups. Notably, there was no statistically substantial difference ($P2 = 0.621$) between the NaF and control groups. Conversely, a highly statistically significant difference ($P2 < 0.001$) was observed between the NaF and NaF+Ga groups (Table 4).

As shown in Table 5, in relation to SOD, the levels exhibited an increase in the NaF group (0.4 ± 0.05 U/mL) in comparison to both the NaF+Ga group (0.37 ± 0.03 U/mL) and the CN (normal) group (0.33 ± 0.01 U/mL). These differences were found to be highly st9atistically significant ($P < 0.001$) across all three groups, specifically between the NaF and control groups. No statistically substantial differences ($P=0.064$) were observed between the NaF and NaF+Ga groups.

In terms of CAT, the levels were observed to be higher in the NaF group (5.51 ± 0.28) in comparison to both the NaF+Ga group (3.58 ± 0.36 U/mg) and the control group (1.65 ± 0.45 U/mg). These differences were found to be highly statistically significant ($P < 0.001$) between all three groups, as well as between the NaF and control groups, and between the NaF and NaF+Ga groups (Table 5).

The activity of glutathione peroxidase (GPx) was found to be reduced in the NaF group (Mean ± SD:35.8 ± 2.4) as compared to both the NaF+Ga group (36.6 ± 2.7 U/mg) and the control group (37.5 ± 3.7 U/mg). However, these differences did not reach statistical significance ($P > 0.05$)

Table 3. Comparison of all studied groups as regards cardiac enzymes and lactate dehydrogenase (LDH)

	Experimental groups			*P* value
	CN	NaF	NaF+Ga	
Total CK (U/L)	125±1.7	741±16	341±8	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
CK MB (IU/L)	280±1.7	458±1.6	301±2.3	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
cTN1 (ng/mL)	0.34±0.03	0.76±0.06	0.36±0.17	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
LDH (U/L)	360±3.2	540±3.1	398±9.4	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**

*P*1= *P* value of significance between all three groups. *P*2 = *P* value of significance between CN (control group) and NaF groups. *P*3 = *P* value of significance between NaF (sodium fluoride) and NaF+Ga groups. **P* value < 0.05 is considered significant; ***P* value <0.001 is considered highly significant.

Table 4. Comparison of all studied groups regarding lipids profile

	Experimental groups			*P* value
	CN	NaF (sodium fluoride)	NaF+Ga	
TC (mg/dL)	75±0.8	135±1.4	90±1.4	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
TG (mg/dL)	76.7±1.7	105±1.7	91±2	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
HDL (mg/dL)	43.3±1.2	31.6±2.1	38.3±2.1	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
LDL (mg/dL)	35±11.2	36.8±2.4	24.9±1.6	*P*1= 0.001*, *P*2= 0 621, *P*3<0.001**

*P*1= *P* value of significance between all three groups. *P*2 = *P* value of significance between CN (control group) and NaF groups. *P*3 = *P* value of significance between NaF (sodium fluoride) and NaF+Ga groups. **P* value < 0.05 is considered significant; ***P* value <0.001 is considered highly significant.

Table 5. Comparison of all studied groups as regards oxidative stress biomarkers

	Experimental groups			*P* value
	CN	NaF (sodium fluoride)	NaF + Ga	
SOD (U/mL)	0.33±0.01	0.4±0.05	0.37±0.03	*P*1<0.001**, *P*2<0.001**, *P*3= 0.064
CAT (U/mg)	1.65±0.45	5.51±0.28	3.58±0.36	*P*1<0.001**, *P*2<0.001**, *P*3<0.001**
GPx (U/mg)	37.5±3.7	35.8±2.4	36.6±2.7	*P*1 = 0.45, *P*2 = 231, *P*3 = 0.466
GSH (mmol/L)	74.5±1.6	72±1	73.2±1.2	*P*1 = 0.001*, *P*2 = 0.001*, *P*3 = 0.02*

*P*1= *P* value of significance between all three groups. *P*2 = *P* value of significance between CN (control group) and NaF groups. *P*3 = *P* value of significance between NaF (sodium fluoride) and NaF+Ga groups. **P* value < 0.05 is considered significant; ***P* value <0.001 is considered highly significant. P-value >0.05 is considered non-significant. SOD, Superoxide dismutase; CAT, Catalase; GSH, Glutathione; GPx, Glutathione peroxidase.

across all three groups, as well as between the NaF and control groups, and the NaF and NaF+Ga groups.

In relation to GSH levels, a statistically significant decrease (P= 0.001) was observed in the NaF group (72 ± 1 mmol/L) compared to both the NaF+Ga group (73.2 ± 1.2 mmol/L) and the control group (74.5 ± 1.6 mmol/L). This difference was statistically significant among all three groups, as well as between the NaF and control group. Furthermore, a statistically significant (P= 0.02) difference was noted between the NaF and NaF+Ga groups (Table 5).

Discussion

Hypertension has the potential to induce cardiac tissue remodeling and contribute to renal impairment. Some antihypertensive interventions may yield adverse effects and modify the disease's clinical trajectory (17). Consequently, identifying natural and safe alternative therapies is of paramount importance. Effective management and supervision of hypertension are critical in mitigating cardiovascular disease and its associated complications (18). A recent investigation evaluated the impact of garlic on NaF-induced hypertension and elevated cardiac enzymes in male albino rats. The findings demonstrated that garlic significantly reduced arterial blood pressure and conferred cardiovascular protection in the hypertensive rats (11).

Cardiovascular disease stands as a leading cause of mortality, with a notable absence of specific therapeutic alternatives to address clinical needs. TNF-α, a key pro-inflammatory cytokine, significantly impacts various immunopathogenic processes (19).

The part of the garlic that is responsible for its biological activity is the active organosulphuric compounds, which include allicin, alliin, diallyl sulfides (DAS), and diallyl trisulfide. Many studies have shown that garlic compounds have antioxidant, antimicrobial, antimutagenic and anticancer properties. Subedi et al (20) found that DAS reduces TNF-α in rat aortic smooth muscles and suggested that DAS could prevent oxidative stress in inflammation. Furthermore, immunohistochemical studies have indicated that DAS reduces inflammatory biomarkers, such as TNF-α and IL-1β, by affecting inducible nitric oxide synthase (iNOS) and activating nuclear factor kappa B (NF-κB). Another study demonstrated that allicin inhibits NF-κB activation as well as TNF-α and iNOS production. Likewise, it was shown that DAS inhibits both pro- and anti-inflammatory cytokines in LPS-stimulated macrophages (21). Histological analysis of the NaF group demonstrated significant deformation, fragmentation, and loss of striation in heart muscle. It is known that NaF causes severe damage to liver tissue (22) and heart (6,22). Garlic has been proven to greatly affect the histology of the cardiac muscles, which were affected by NaF, resulting in the preservation of cardiomyocyte morphology, arrangement, and tissue space, which were more or less similar to the control group. The meta-analysis conducted by Imaizumi et al (23) revealed that garlic had been shown in trials to lower blood pressure, waist circumference, body mass index, LDL, HDL, TC, TG, and inflammatory markers. It can also raise HDL levels and improve cardiovascular parameters like coronary artery calcium, microcirculation, epicardial and periaortic adipose tissue, post occlusive reactive hyperemia, low attenuation plaque, carotid intima-media thickness, and carotid intima-media thickness (23). For these reasons, garlic should be considered to prevent and treat cardiovascular disease risk factors. Garlic's action on high blood pressure involves allicin compounds, which dilate blood vessels, enhance NO availability, and have cardiovascular-protective antioxidant properties (24). Consuming garlic in supplements can normalize high blood pressure, with a low incidence of side effects (25). It can be used in conjunction with other lifestyle interventions for improved blood pressure regulation. Mechanisms of antihypertensive action of garlic are related to its prostaglandin-like effects, which decreases peripheral vascular resistance (26). Garlic reduce prostaglandin E2 and thromboxane B2 level and thereby can reduce hypertension. The gamma-glutamyl cysteines are the compounds in garlic, they inhibit angiotensin-converting enzyme and for this mechanism, garlic can lower blood pressure (27). Garlic also inhibited endothelin-1 induced contraction in a dose-dependent manner. Allicin and ajoene in Garlic appear to inhibit iNOS in macrophages, reducing nitrite accumulation in atherosclerotic plaques and in hypoxic tissues (28). The NaF+Ga co-treated group showed few blue-stained fibers and a statistically significant decrease in its percentage area compared with the NaF group. This finding is consistent with Mohamad and colleagues' findings, as the amount of collagen fibers (elastic fibers) was reduced in the group administered garlic (29). Garlic possesses a multitude of biological benefits, such as its ability to prevent the formation of cancer cells, its capacity to neutralize harmful substances in the body, its ability to combat microbial infections, its ability to prevent genetic mutations, its capacity to alleviate asthma symptoms, its ability to modulate the immune system, and its ability to promote the growth of beneficial bacteria in the gut (30, 31). The substances derived from it, such as allicin, alliin, and allyl methyl trisulfide (MATS), can decrease levels of inflammatory indicators by controlling the activity of IL-10 and NF-κB. This protein complex can alter the regulation of genes associated with inflammation (32). Garlic is abundant in vitamins such as B complex vitamins and vitamin C, which makes it a food that is rich in nutrients and has functional benefits. Recent research indicates that garlic may have promising preventative benefits on risk factors associated with cardiovascular disease (33). Supplementing with aged garlic extract has been demonstrated to alter elevated blood TNF-α and IL-6 levels in overweight people experiencing mild inflammation (34). Nevertheless, certain experimental

investigations have indicated that adding garlic does not change the levels of TNF-α and high-sensitivity C-reactive protein in the bloodstream, although other studies also have discovered no impact on IL-6 and TNF-α (35). Exposure to NaF disturbs the delicate equilibrium between pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) proteins, resulting in an imbalance that favors cell death (apoptosis) in the myocardium. It appears that NaF harms heart tissue (36). Co-treating with garlic has a mitigating effect on this. Through the modulation of Bcl-2 and Bax expression, garlic demonstrates its ability to safeguard against NaF-induced apoptosis, thereby supporting cell survival in the myocardium (37). The significant increase in all four biomarkers in the NaF group strongly suggests that exposure to NaF causes harm to heart muscle cells. This is in line with previous findings showing higher Bax levels and lower Bcl-2 levels, indicating a process of cell death through apoptosis (38). The group that received NaF+GA exhibited significantly lower levels of all four biomarkers compared to the group that only received NaF. The combination of garlic with NaF could potentially provide protection against muscle damage.

Asdaq et al (39) discovered that animals given a high dose of garlic oil [100 mg/kg, per oral (p.o.)] experienced a noticeable reduction in CK levels, indicating the protective role of garlic against cardiac muscle damage (39). The research findings demonstrate that exposure to NaF disrupts normal blood lipid profiles, resulting in elevated levels of TC, TG, and LDL, thereby posing an increased risk of cardiovascular issues. The reduction in HDL further compromises the body's ability to eliminate LDL from the bloodstream. Notably, the group treated with both NaF and garlic exhibited significantly decreased levels of TC and TG, along with elevated levels of HDL, in comparison to the group treated with NaF alone. These results suggest that the administration of garlic alongside NaF can ameliorate the adverse effects of NaF on blood lipid profiles by reducing TC, TG, and LDL while simultaneously increasing HDL. This has the potential to mitigate the cardiovascular risk associated with NaF exposure.

In the study conducted by Zhao et al (40), it was observed that the consumption of garlic led to a reduction in lipid parameters within the study group. The researchers noted a significant enhancement in TC, LDL and HDL levels among the group that consumed garlic in comparison to the group that received a placebo (40). The study findings indicate that exposure to NaF disrupts bodily antioxidant defence, leading to heightened SOD and CAT activity. In the NaF+Ga group, GSH levels were notably elevated compared to the NaF group, with no significant alterations in SOD, CAT, or GPx activity. This suggests that garlic may contribute to maintaining GSH levels, a critical antioxidant molecule, and may offer some defense against NaF-induced GSH depletion (41). The NaF+Ga group exhibited levels akin to those of the control group but with elevated GSH levels. Although the precise mechanism underlying this effect remains incompletely understood, it does appear to provide some defense against GSH reduction caused by NaF (42). This study is subject to certain limitations, notably the use of a low-dose to induce hypertension in rats. Additionally, the duration of the garlic treatment was only four weeks, which is considered a relatively short exposure period. Extending the exposure time may yield varying effects on the parameters under investigation. Further investigations are needed on both male and female rats. Different doses of NaF and garlic should be applied to study their effects on the parameters.

Conclusion

The presence of NaF has been linked to adverse effects on the cardiovascular system. In recent guidelines advocating for the exploration of natural remedies to manage hypertension, garlic has emerged as a subject of interest due to its potential to reduce blood pressure. Consequently, it is plausible to posit that garlic may mitigate NaF-induced cardiac dysfunction and lipid profile alterations in rat models.

Authors' contribution

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical issues

This study was conducted in accordance with all experimental protocols approved by the Research Ethics Committee of the Faculty of Medicine for Girls at Al-Azhar University (FMG-IRB) (ethical code #IRB 2379). It adhered to the 'Guide for the Care and Use of Laboratory Animals' as outlined by the National Institutes of Health (NIH, 1978). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Data Availability statement

Data will be available on request from the corresponding author.

Funding/Support

No funding.

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