THE EFFECT OF RESVERATROL THERAPY ON THE VASCULAR RESPONSES CAUSED BY CHRONIC FLUOROSIS

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ABSTRACT: The aim of this study was to investigate the effect of resveratrol therapy on the contraction-relaxation responses of the thoracic aorta rings and on the blood pressure of rats exposed to chronic fluorosis. The study was conducted using 80 male and female Sprague Dawley rats weighing 200–250 g. The rats were randomly divided into 8 same gender groups of 10 (4 groups of male rats and 4 of female rats) and administered, for each gender via the drinking water, (i) 0 mg/L of sodium fluoride (NaF) and 0 mg/L of resveratrol (control groups); (ii) 10 mg/L of NaF; (iii) 50 mg/L of resveratrol; and (iv) 10 mg/L of NaF and 50 mg/L of resveratrol. Blood pressures were measured on days 30, 60, and 90 in each group. At the end of day 90, the contraction-relaxation responses of the thoracic aorta rings were examined in an isolated organ bath. The analysis on the serum fluorine level revealed that the serum fluorine level was elevated in all the rats administered NaF. For each gender, the most marked elevations in the blood pressures were seen, on all three days, in the NaF groups. In both the male and female groups, the chronic administration of resveratrol with NaF led to decreased blood pressures. The contraction response resulting from phenylephrine administration was increased in the groups administered NaF, whereas it was decreased in the groups administered NaF+resveratrol. In the groups administered resveratrol, the maximal relaxing effect was achieved by acetylcholine. In the groups administered NaF, the relaxation responses caused by SNP (sodium nitroprusside) were similar to those in the other study groups. The results of this study indicate that resveratrol provides a protective effect against the increased blood pressure caused by NaF and the potential endothelial damage. The protective effect of resveratrol results from its capability to reduce fluorine-induced oxidative stress and endothelial tissue damage. Further studies are warranted to investigate the adverse effects in the veins exposed to chronic fluorosis and the molecular mechanisms of the potential protective effects of resveratrol against these adverse effects.

Key words: Blood Pressure; Contraction responses; Fluorine; Resveratrol; Thoracic aorta.

1. INTRODUCTION

Endemic fluorosis resulting from high fluoride concentration in groundwater is a public health problem in the many countries, including India, which lie in the geographical fluoride belt that extends from Turkey to China and Japan through Iraq, Iran, and Afghanistan.[1] Fluoride in drinking water should be below a certain concentration range for human health. The upper limit for fluoride in drinking water recommended by the World Health Organization (WHO) in 1984 is 1.5 ppm[2] but this has been seen to be unsuitable in some countries and lower Country Standards have been set for some countries such as in India of 1 mg/L where there is a rider that the "lesser the fluoride the better, as fluoride is injurious to health," and Senegal, West Africa, of 0.6 mg/L.[3]

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Chronic fluorosis occurs when natural fluorine compounds are taken in excess for a long time. In the formation of chronic fluorosis, naturally occurring fluorine in soil, water, and plants plays an important role. Industrial activities related to fluorine[4] and volcanic regions[2] increase the fluorine ratio of natural products.

Approximately 80% of the fluorine taken into the organism is absorbed from the gastric and intestinal mucosa by simple diffusion.[5] Absorption through the lung is much faster than through the gastrointestinal tract.[6] The plasma fluoride concentration with a standard fluoride uptake ranges from 0.5–1.0 μM. Ninety % of the fluorine passing into the bloodstream is bound to the protein while the remaining 10% is in the ionized form. Fluoride is rapidly dislodged from the plasma and replaced with anions (hydroxyl, citrate, and carbonate) in bone and tooth tissues, resulting in approximately 95% fluoride accumulation in these tissues.[5] Ninety% of the excretion of fluoride is via urine. In addition, sweating and milk loss are insignificant.[7] When fluoride is ingested by humans, 90% is absorbed into the blood stream and 10% is excreted in faeces. Nearly half of the absorbed fluoride is quickly taken up by bone and growing teeth, about 45% is filtered in the kidneys and excreted in urine, and approximately 5% is excreted in breast milk, sweat, and saliva.[8]

Acute or chronic overexposure of the organism to fluorine compounds causes pathological conditions in many systems. Epidemiological studies with electrocardiograms of dental fluorosis sufferers show that 12.8% had reduced myocardial function and 29.5% had abnormal heart rhythms.[9] Another study showed that 50–73% of patients with skeletal fluorosis had abnormal electrocardiograms with a clear demonstration of an increase in abnormal heart rhythms and signs of myocardial damage.[10]

Although the toxic effect of fluoride on the heart and arteries has been examined by many researchers and carefully reviewed, it has not attracted the interest of science journalists or the media, and therefore the general public and politicians remain ignorant of the risks.[11] Fluoridation is known to increase systolic blood pressure.[12] This hypertension is a primary risk factor for cardiovascular disease. Vascular smooth muscle contractions with NaF exposure occur due to activation of the Rho-kinase pathway.[13]

Studies in humans and animals show that chronic fluoride exposure causes oxidative damage[14,15] that promotes inflammatory mechanisms, vascular stiffness, atherosclerosis, and myocardial cell damage.[16,17]

Fluoride poisoning can be avoided or minimized by reducing fluoride intake by using alternative water resources with a low fluoride content and avoiding high fluoride foods.[18]

Resveratrol is a polyphenolic phytoalexin that is synthesized by plants in response to traumatic injury or fungal attack. Phytoalexin is an antibiotic of plants produced by any external stress or pathogenic attack.[19] Resveratrol is the main component of a molecular family that contains glycosides and polymers, and is found in high amounts in vegetables and fruits, such as peanuts, raspberries, plums, berries, blackberries, and grapes, and in red wine.[20] Recent studies have shown that resveratrol, with its antioxidant properties, has beneficial effects on the organism and can be used in the prevention and treatment of some metabolic diseases.[22]

Resveratrol increased resistance to stress and prolonged the survival of many organisms from yeasts to vertebrates. It has cardiovascular protective activity via its vasodilatory,[22,23] antihyperlipidemic, anti-inflammatory, antiaggregant, and antioxidant[23,24] properties. It is thought that the protective effects of resveratrol on the cardiovascular system may be due to its antioxidant properties, its effects on the regulation of lipid metabolism, its increasing cellular NO levels, and its inhibiting platelet aggregation.[25]

Studies on the relationship between endemic fluorosis and gender are limited. Kotecha et al.[24] reported that the prevalence of fluorosis was greater in males (61.30%) as compared to females (57.26%). On the other hand, the results of a different study did not show any association between the occurrence of fluorosis and sex.[27]

In this study we investigated the effect of NaF and resveratrol on contraction-relaxation responses in blood pressure and thoracic aortic rings in male and female rats who were subjected to chronic fluorine intake.

2. MATERIALS AND METHODS

2.1. Animals: All experiments were performed on 80 adult male and female Sprague-Dawley rats weighing 200–250 g from the animal experiment center of Van Yüzüncü Yıl University. Animals were housed in a well-ventilated and air-conditioned area provided with independently adjustable light-dark cycle (12 hr light/ 12 hr dark cycle) and temperature regulation systems. Temperature was maintained at 22 ± 2ºC and humidity was kept at 45–70%. The rooms and animal cages were cleaned daily and the animals were provided with fresh food and water *ad libitum* on a daily basis. This study was approved by Van Yüzüncü Yıl University Experimental Animals Local Ethics Committee (YUHADYEK 28.01.2016/ Decision no: 2016/01).

2.2. Groups: The rats were randomly divided into 8 same gender groups of 10 (4 groups of male rats and 4 of female rats) and administered, via the drinking water, (i) 0 mg/L of sodium fluoride (NaF) and 0 mg/L of resveratrol (control groups); (ii) 10 mg/L of NaF; (iii) 50 mg/L of resveratrol; and (iv) 10 mg/L of NaF and 50 mg/L of resveratrol. The rats were treated with NaF and resveratrol for 90 days in drinking water and the study was terminated at the end of the 90th day.

2.3. Chemicals: The chemicals used were: resveratrol (R5010), sodium fluoride (S7920), sodium nitroprusside (71778), acetylcholine, phenylephrine, NaCl (S7653), KCl (P9541), $MgCl.6H_2O$ (M2670), NaH_2PO_4 (S8282), $CaCl_2.2H_2O$ (C5080), $NaHCO_3$ (S5761), dextrose (G7021), ketamine (K-002), and xylazin (X1126).

2.4. Serum fluorine analysis: During measurement of the fluoride ion activity, the total ionic strength adjustment buffer (TISAB) was used to keep the total ionic strength of the solution constant, to adjust the pH, and to degrade the fluorine ion complexes with metal cations such as aluminum, iron, and magnesium.

Blood samples taken from rats were centrifuged at 4ºC, 3,000 rpm for 15 minutes, and the serum was removed. One mL of TISAB II solution was added. The combined pH and the combined selective fluorine electrode were immersed in the mixture to check that the pH was between 5.0 and 5.5. The measurement is complete when the reading is fixed. The fluorine electrode and ppm values were read.[28]

2.5. Blood pressure recordings: Blood pressure measurements were made by the tail sleeve method using the tail cuff device while the rats were conscious. Blood pressure measurements of rats were calculated at 30, 60, and 90 days and by 3 measurements from each rat. Each rat was placed alone in a restrainer box and the cuff and sensor of the tail-cuff device were then placed on the rat's tail. The tails of the rats were heated to 37ºC for 1–20 minutes until they received a regular signal tone and pulse. The measurements were performed in a quiet and calm laboratory environment, where the rats were comfortable and calm, and, when there was a regular signal sound, the values were recorded on a computer.

2.6. Isolated organ bath experiments: At the end of the study, after the midline abdominal incision of the rats under ketamine anesthesia, the cartilage on both sides of the sternum was cut and the thoracic cavity was accessed. The thoracic aorta was dissected by gently dissecting just below the aortic arch and taken to the petri dish with Krebs solution. Transverse rings of the thoracic aorta were cleaned from the surrounding fat and connective tissue and prepared as 3 to 4 mm long strips. Care was taken to avoid damage to the endothelial layer during the preparation of the preparation. The thoracic aortic rings was suspended at 37ºC with 5% CO_2 and 95% O_2 in a medium containing 15 mL normal Krebs solution (NaCl 112 mM, KCl 5 mM, $MgCl.6H_2O$ 0.5 mM, NaH_2PO_4 1.2 mM, $CaCl_2.2H_2O$ 2.5 mM, $NaHCO_3$ 25 mM, dextrose 11.5 mM, and pH: 7.4). Appropriately prepared clips of fine stainless steel were passed through the lumens of the annular aortic preparations. One of the two clips passing through the lumen of each ring was attached to the hook of the organ bath to secure the vessel and the other was attached to the isometric transducer using an appropriate length of thread. After the aortic rings were placed in the organ bath, they were allowed to rest for 45 minutes under a resting tension of 2 g, which is the optimal passive tension for the rat aorta.

The Krebs solution in the baths where the tissues were contained was replaced with fresh Krebs solution every 15 minutes. At the end of this period, 10^{-6} M phenylephrine was added to the arterial rings and contractions were obtained. After each contraction, the aortic rings were washed with Krebs solution for 15 minutes and allowed to rest for 45 minutes. At the end of this period, cumulative concentrations of ACh ($10^{-8, -7, -6, -5}$) and SNP ($10^{-10, -9, -8, -7, -6, -5}$) were given to the phenylephrine contracted vessels and the relaxation responses were recorded.

2.5 Statistical analysis: The descriptive statistics in our study are expressed as: mean, standard deviation, median, and minimum and maximum values. The Mann-Whitney U analysis was performed to compare the group averages. The Duncan test was used to compare the means in different groups. In addition, the Wilcoxon test was used to calculate the difference between the time periods in the same group. The statistical significance level was taken as 5% in the calculations and the SPSS (version 20) statistical package program was used for the calculations.

3. RESULTS

Blood pressure analyzes were performed in the male and female rats on the 30^{th}, 60^{th}, and 90^{th} days of the study. At the end of the study, the levels of fluorine in the serum samples, obtained from the blood taken from rats, and the contraction relaxation responses of the thoracic aortic rings of the rats were examined.

3.1 Serum fluoride analysis: The results of the fluoride analysis in the serum samples taken from rats are shown in Table 1.

Table 1. The levels of fluoride in the serum (ppm) (NaF = sodium fluoride, Res = resveratrol, Std. Dev = standard deviation, Min = minimum, Max = maximum)

Group	Male rats			Female rats		
	Mean ± Std. Dev.	Min	Max	Mean ± Std. Dev.	Min	Max
Control	0.0073±0.0005[a]	0.007	0.008	0.0080±0.0009[a]	0.007	0.009
NaF	0.0118±0.0008[b]	0.011	0.013	0.0127±0.0010[b]	0.011	0.014
Res	0.0085±0.0008[a]	0.007	0.009	0.0083±0.0008[a]	0.007	0.009
Res+NaF	0.0113±0.0016[b]	0.009	0.013	0.0108±0.0010[c]	0.009	0.0012

[a,b,c]: At the same measurement time, the difference between the group averages of different lowercase letters is significant ($p<0.05$).

The fluoride levels in both the male and female sodium fluoride (NaF)-treated rats were significantly higher than in the corresponding control group ($p<0.05$). The serum fluoride levels in the resveratrol (Res) group were not significantly different to the control values ($p>0.05$). The serum fluoride levels in the female Res+NaF group were significantly lower ($p<0.05$) than those in the female NaF group, but no significant difference was found in the corresponding groups for male rats ($p>0.05$).

3.2 Blood pressure: The results of blood pressure analyzes in the rats are shown in Table 2 and Figures 1–5. For all the measurement times (30th, 60th, and 90th days) in both the male and female rats, the group with the most significant increase in blood pressure was the NaF group.

Considering the group averages for the blood pressure in the male rats on the 30th day, the blood pressure increase in the NaF group showed a significant increase ($p<0.05$) compared to the control group and the group treated with Res+NaF. On the 60th day, the increase in blood pressure in both the NaF and the Res+NaF groups of male rats was significantly higher ($p<0.05$) than in the control group and the Res group. On the 90th day, the blood pressure values were significantly higher ($p<0.05$) in the NaF group ($p<0.05$) compared to the control, Res, and Res+NaF groups and this level was also higher than that in the male NaF group rats on the 30th and 60th days.

For the female rats, the blood pressure values measured on the 30th day were significantly higher in the NaF, Res, and Res+NaF groups ($p<0.05$) compared to control. On the 60th day, the increase in blood pressure in both the NaF and the Res+NaF groups of female rats was significantly higher ($p<0.05$) than in the control and the Res groups. On the 90th day, the blood pressure values in the NaF group were

significantly higher than those in the other 3 groups ($p<0.05$) and was higher that than in the female NaF group rats was on the 30th and 60th days.

Comparing the groups by gender on the 30th day, the blood pressure was significantly different ($p<0.05$) in the control and Res+NaF groups. Comparing the groups by gender on the 60th day, the blood pressure was significantly different ($p<0.05$) in the Res+NaF group. Comparing the groups by gender on the 90th day, the blood pressure was not significantly different in any of the groups.

Table 2. Descriptive statistics and comparison results for blood pressure (mm/Hg)

Day	Group	Male rats			Female rats		
		Mean±Std. Dev.	Min	Max	Mean±Std. Dev.	Min	Max
30th day	Control	124.75±7.50$^{A,b,\#}$	118.0	137.0	117.75±1.58^{B,C,b}	115.0	119.0
	NaF	141.25±15.55^{B,a}	116.0	164.0	141.25±15.78^{C,a}	114.0	166.0
	Res	131.38±10.72^{A,B,a,b}	118.0	143.0	139.25±29.78^{A,a}	91.0	174.0
	Res+NaF	121.63±18.07$^{A,b,\#}$	100.0	149.0	139.00±6.59^{B,a}	126.0	148.0
60th day	Control	120.88 ±14.36^{A,b}	102.0	139.0	118.88±4.19^{B,b}	114.0	127.0
	NaF	149.25 ±20.82^{B,a}	118.0	171.0	157.13±7.95^{B,a}	145.0	170.0
	Res	132.75 ±7.72^{A,b}	121.0	142.0	119.25±17.25^{A,b}	95.0	151.0
	Res+NaF	133.88±15.23$^{A,a,\#}$	115.0	160.0	155.50±18.69^{A,a}	134.0	179.0
90th day	Control	131.34 ±6.81^{A,b}	119.9	140.4	127.40±4.55^{A,b}	122.1	136.2
	NaF	171.1 ±8.73^{A,a}	158.0	182.6	173.88±8.93^{A,a}	158.5	183.1
	Res	122.32 ±10.06^{B,b}	109.2	137.5	120.84±10.59^{A,b}	109.5	135.3
	Res+NaF	126.68 ±11.94^{A,b}	105.8	140.6	127.26±13.06^{B,b}	105.9	143.8

^{a,b,c}: On the same measurement day, the difference between the group averages with different lower case letters is significant ($p<0.05$).

^{A,B,C}: In the same group on different measurement days, the difference between the group averages with different upper case (capital) letters is significant ($p<0.05$).

$^{\#}$: In the same group, apart from gender differences, on the same measurement day, the gender difference is significant ($p<0.05$).

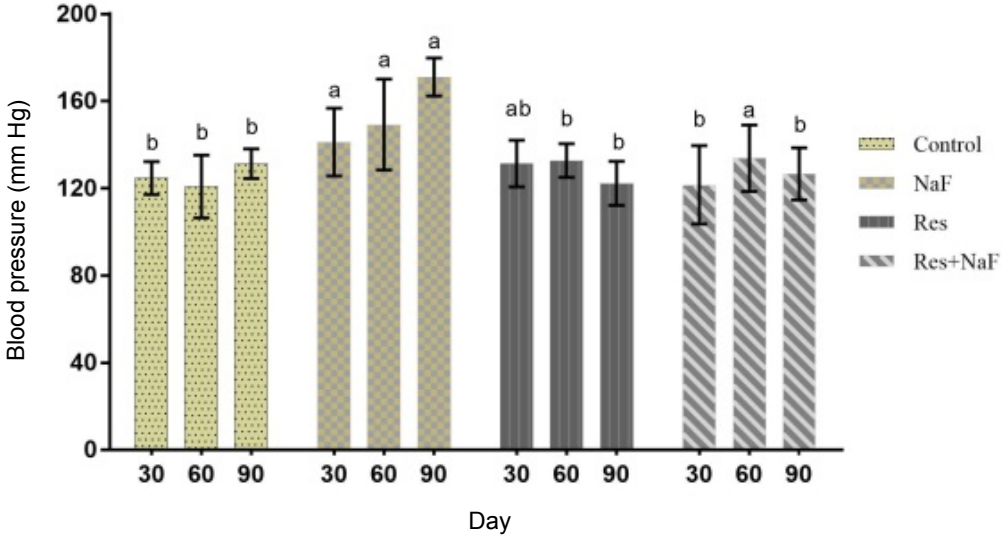

Figure 1. The results of blood pressure measurement (mm Hg) in male rats on the 30th, 60th, and 90th days.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

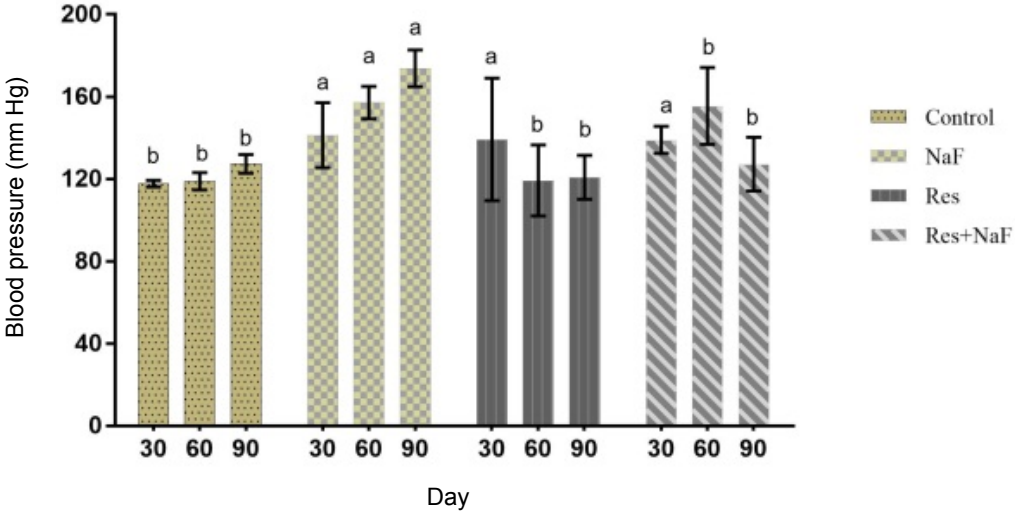

Figure 2. The results of blood pressure measurement (mm Hg) in female rats on the 30th, 60th, and 90th days.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

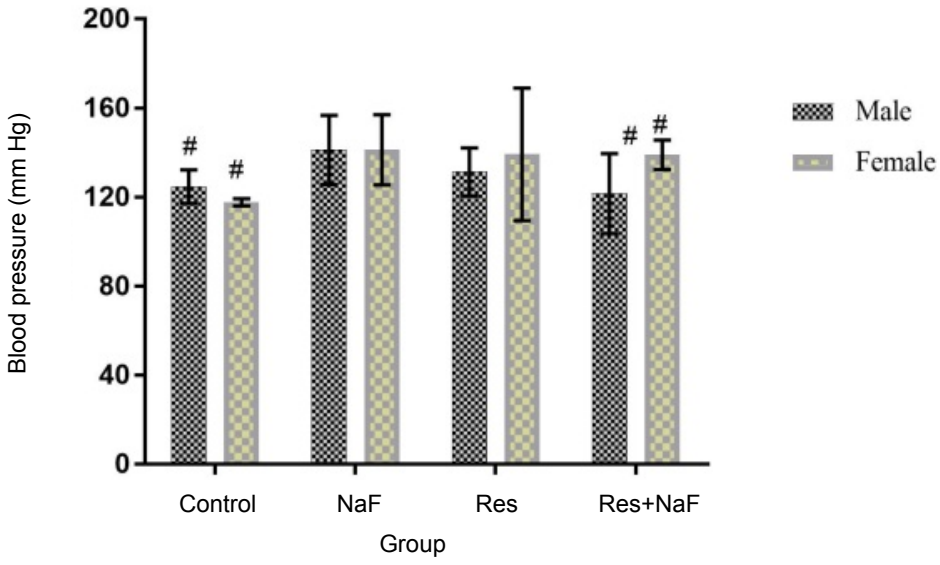

Figure 3. The results of blood pressure measurement (mm Hg) in male and female rats on the 30th day.
#: The gender difference is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

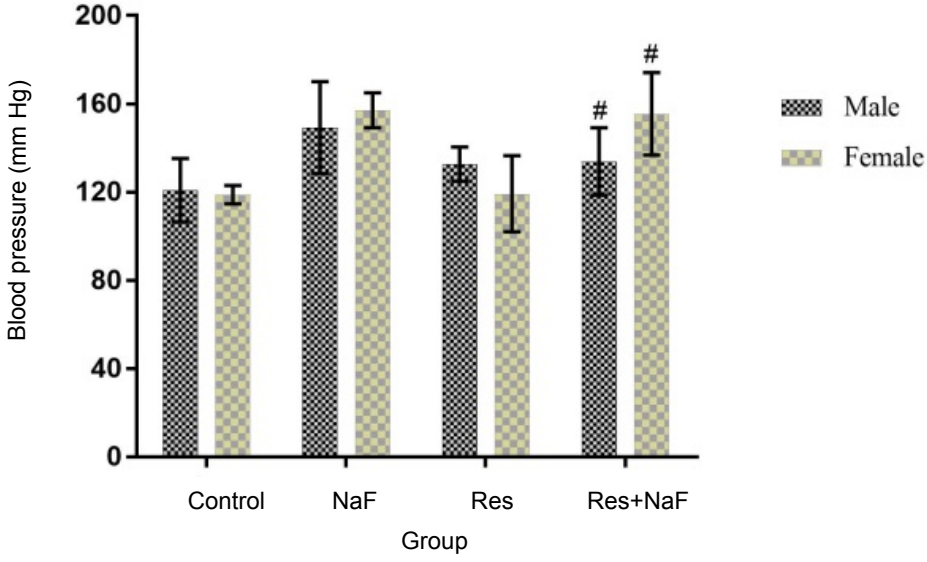

Figure 4. The results of blood pressure measurement (mm Hg) in male and female rats on the 60th day.
#: The gender difference is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

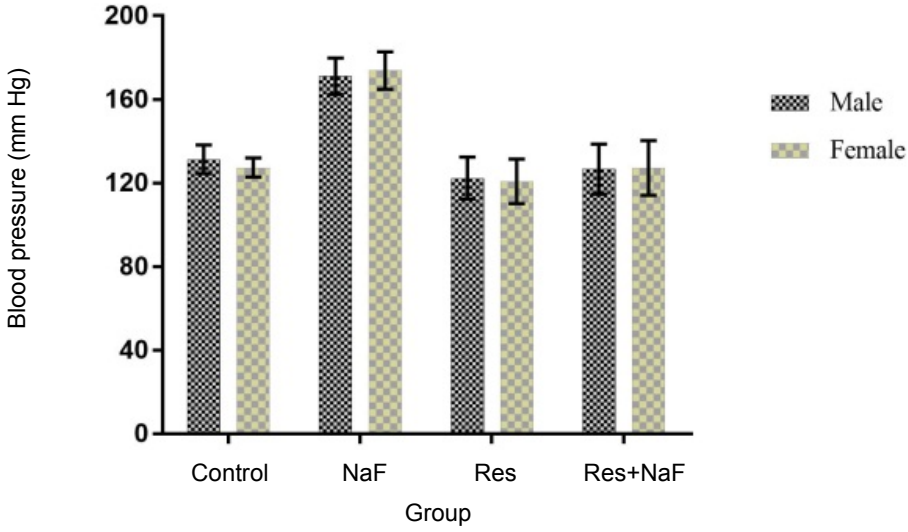

Figure 5. The results of blood pressure measurement (mm Hg) in male and female rats on the 90th day.
#: The gender difference is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

3.3. Experiments with aortic rings in an isolated organ bath: In this study, the effects of different acetylcholine (ACh) and sodium nitroprusside (SNP) levels on the contractions of endothelial (+) vascular smooth muscle induced with phenylephrine were studied.

3.3.1 Contraction responses to phenylephrine: The addition of 10^{-6} M phenylephrine to Krebs baths resulted in phenylephrine-induced contractions in all the groups.

In the male rats, the contractile response after the phenylephrine application was found to be significantly greater in the NaF group compared to the other groups ($p<0.05$ (Figure 6).

In the female rats, the contractile response observed after the administration of phenylephrine was found to be significantly greater ($p<0.05$) in the NaF group compared to the other groups and in the Res+NaF group compared to the Res group (Figure 7).

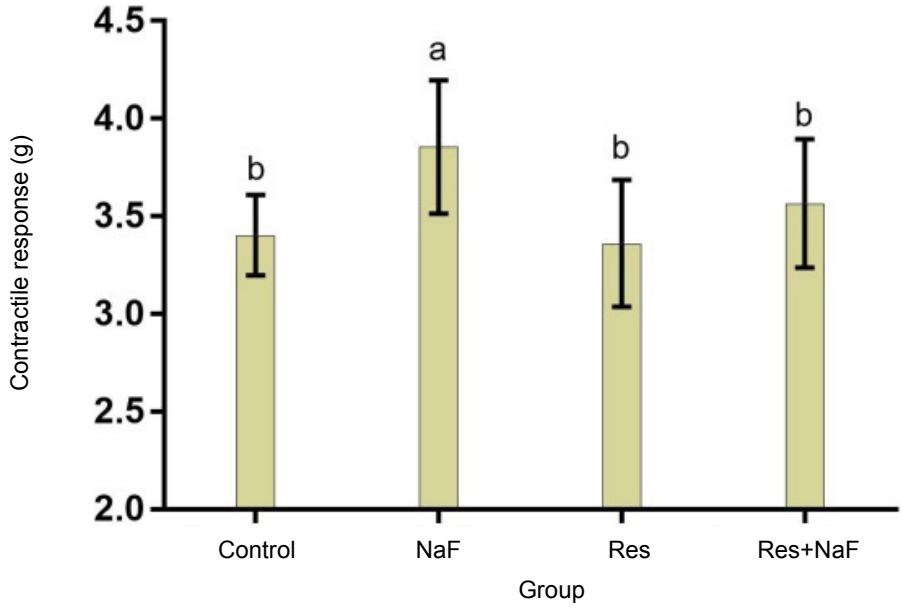

Figure 6. The contractile responses (g) to the first dose of phenylalanine (Phe) in the male rats.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

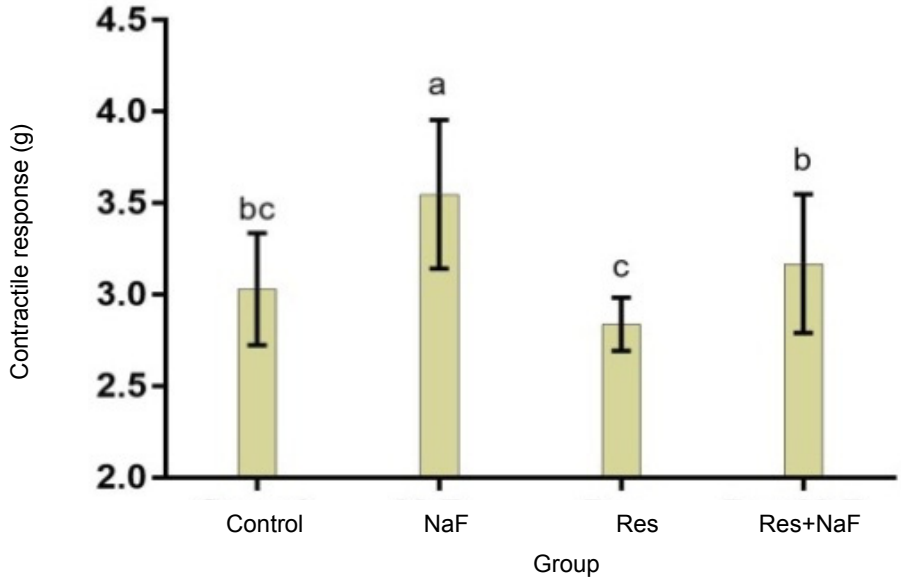

Figure 7. The contractile responses (g) to the first dose of phenylalanine (Phe) in the female rats.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

3.3.2. Effect of acetylcholine: Acetylcholine, which triggers endothelium-dependent relaxation, caused a concentration-dependent (ACh, $10^{-8-7-6-5}$ M) relaxant effect on the rat thoracic artery rings.

In the male rats, the maximum relaxant effect achieved with the acetylcholine was found in the Res+NaF group with a level of approximately 89.36%, which was a result close to that of the control group of male rats. The minimum effect was in the NaF group with a level of 81.00% (Figure 8).

In the female rats, the maximum relaxant effect achieved with the acetylcholine occurred in the Res+NaF and Res groups of over 100%. The relaxation response in the control group was determined to be 88% and the least relaxation response was in the NaF group with a rate of 77.17% (Figure 9).

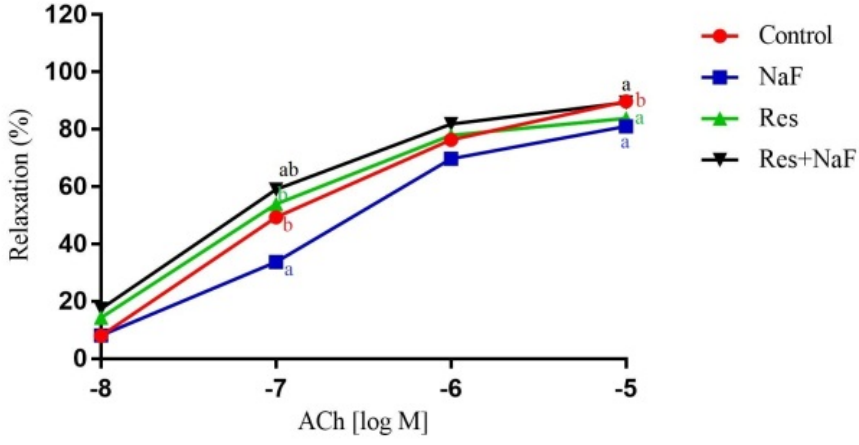

Figure 8. The acetylcholine (ACh) relaxation responses (g) in the male rats.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

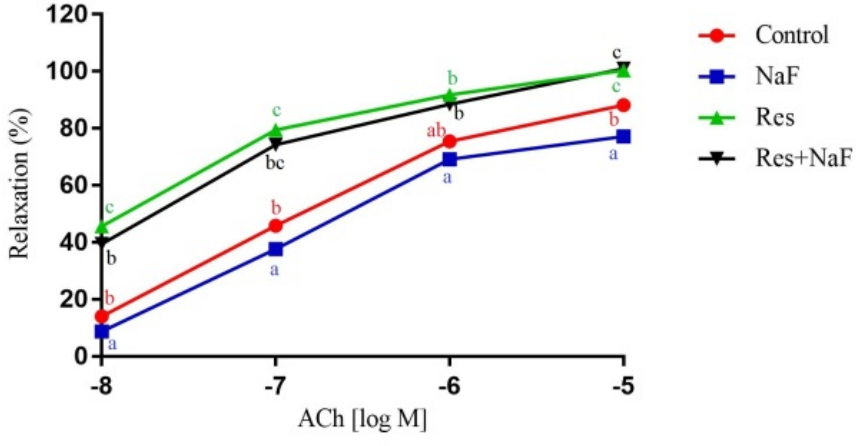

Figure 9. The acetylcholine (ACh) relaxation responses (g) in the female rats.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

3.3.3. Effect of sodium nitroprusside: Sodium nitroprusside (SNP, $10^{9\text{-}8\text{-}7\text{-}6\text{-}5}$ M), which causes relaxation with a direct effect on vascular smooth muscle, caused a contraction-dependent relaxation effect in isolated rat thoracic arteries contracted with Phe.

In the male rats, with 10^{-5} M SNP, the maximum relaxation effect was observed in the Res+NaF group with 109.00%, the minimum relaxation response was found in the control group with a value of 95.15%, and both the Res and NaF groups produced relaxation responses over 100% (Figure 10).

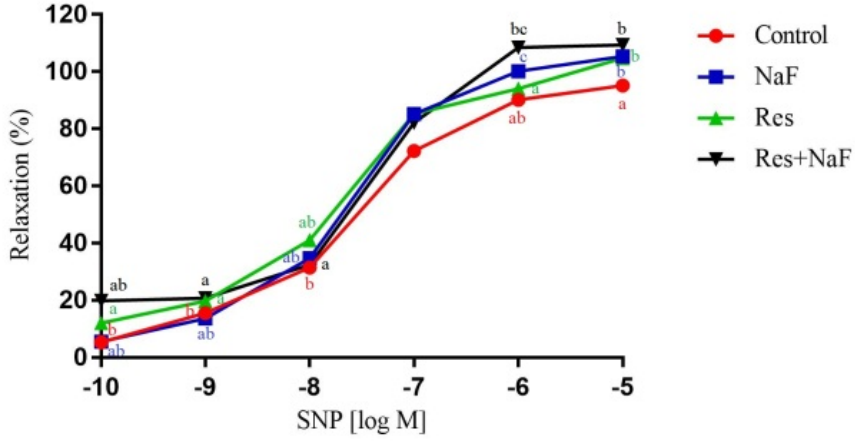

Figure 10. The sodium nitroprusside (SNP) relaxation responses (g) in the male rats.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

In the female rats, with 10^{-5} M SNP, the maximum relaxation effect was observed in the control group with 112.76%. In the Res+NaF group, a relaxation response of 110.06% was obtained. The least relaxation response was determined as 102.39% in the Res group. (Figure 11).

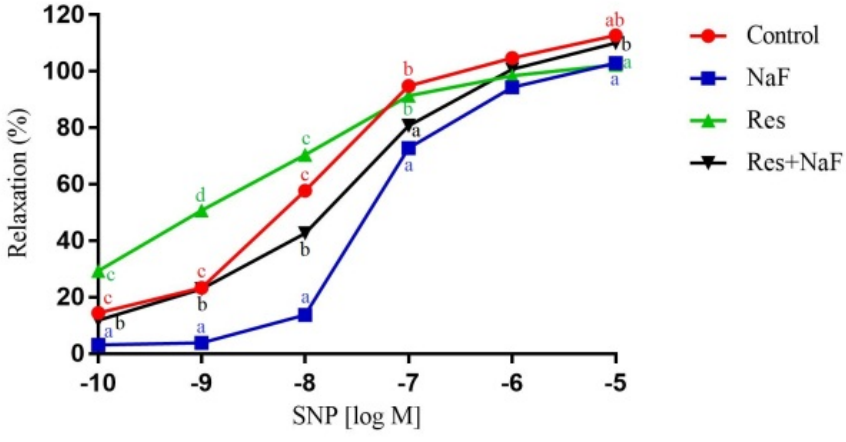

Figure 11. The sodium nitroprusside (SNP) relaxation responses (g) in the female rats.
a,b: At the same measurement time, the difference between the group averages of different lower case letters is significant ($p<0.05$).
Control = control group; NaF = 10 mg/L sodium fluoride group; Res = 50 mg/L resveratrol group; Res+NaF = 50 mg/L resveratrol and 10 mg/L sodium fluoride group.

4. DISCUSSION

In this study, both female rats and male rats were exposed to high doses of fluorine daily for 90 days. At the end of 90th day, the serum fluoride level analysis showed that serum fluoride levels were significantly higher in the NaF and Res+NaF groups compared to the other groups. The Res+NaF groups did not show any increase in the serum fluoride levels compared to the NaF groups suggesting that resveratrol had no effect on the fluorosis levels in the organism.

An experimental study[29] on rats with hypertension found that resveratrol, in a dose of 50 mg/L in drinking water for 12 weeks, reduced the blood pressure. According to the results of this study, the effect of resveratrol has been suggested to be indirectly related to endothelial functions, blood biomarkers, and epigenetic changes. In addition, resveratrol treatment has been reported to reduce fluoride-induced oxidative stress and tissue damage.[30] In the present study, it was observed that the NaF group had the most significant increase in blood pressure in male and female rats at all measurement times (30, 60, and 90 days). In a study by Rush et al.,[31] resveratrol (4.48 ppm) administered for 28 days in drinking water to rats with spontaneous hypertension was reported to cause no significant change in blood pressure. In our study, it was observed that the blood pressure increased at the 30th day in both sex groups receiving resveratrol alone and that, in the female rats, this increase was significant compared to the control group. In both the male and female rats, a reduction in blood pressure was clearly observed when the resveratrol was administered together with NaF, especially on the 90th day. When the results were evaluated according to gender, the blood pressure rises in the Res+NaF groups on the 30th and 60th days were higher in the female rats than in the male rats. On the 90th day, in the Res+NaF groups, both genders had decreased blood pressure and there was no difference between them. These results suggest that chronic use of resveratrol may be effective in reducing increased blood pressure.

Resveratrol is reported to alter the phenylephrine-induced contraction response in isolated rat aorta and to alter fluorine-induced vasoconstriction in isolated coronary arteries.[32] In an experimental study,[33] of noradrenaline- or potassium chloride-contracted mesenteric arteries in male lean and dietary-induced obese rats, resveratrol induced relaxation in both conducting and resistant arteries in a dose-dependent fashion and this was more pronounced in the resistant vessels. Resveratrol has both endothelium-dependent and endothelium-independent relaxing effects in rat aorta,[34] and in rat and guinea pig mesenteric arteries[35] at different concentrations. The endothelium-dependent relaxation effect is reported to be mediated by nitric oxide. Rakici et al.[36] found that resveratrol (70 μM) caused relaxation at a rate of about 35% in the saphenous vein and the internal mammary artery contracted with noradrenaline. It is reported that when the endothelial layer was removed or when L-NOARG (NOS inhibitor) was applied prior to resveratrol administration in the same vessels, the relation due to resveratrol was eliminated.

A study male and female rats, administered resveratrol in drinking water at a dose of 50 mg/L for 21 days, showed that the expression of endothelial nitric oxide synthetase (eNOS) mRNA and protein in the aorta was increased, NO synthesis was improved, and endothelial function was enhanced.[37] In a different study, endothelial cell cultures of bovine pulmonary artery were exposed to resveratrol in 10, 50, and

100 µM concentrations for 3 days.[38] Western blot analysis showed that resveratrol administered at 50 and 100 µM doses caused a 3-fold increase in the expression of eNOS whereas 10 µM resveratrol had no effect on eNOS mRNA expression.[38] In a human study, human umbilical vein endothelial cells (HUVEC) were incubated with 1–100 µg/L resveratrol for 12 to 72 hours.[39] By using a RNAse protection assay, eNOS mRNA expression was found to be increased after 24 hours with a resveratrol concentration of 33 µmol/L. In addition, the eNOS protein level and the eNOS activity were increased in a concentration-dependent manner after 72 hours of resveratrol incubation.[39] The results of these studies indicate that resveratrol can be effective only when used in appropriate doses.

In the present study, the effects of different ACh and SNP levels on the contractions of endothelial (+) vascular smooth muscle induced by phenylephrine were studied. The contractile response seen after phenylephrine administration in both the male and female rats increased only in the group in which NaF was administered compared to the other groups. The contractile response in the Res+NaF groups decreased compared to the NaF-treated groups. It has been reported in different sources that the contractile response increases due to decreased basal relaxation of nitric oxide in cases where the endothelial structure is impaired and nitric oxide (NO) synthesis is decreased.[40] In our study, the increase in the contractile response observed in the NaF group after the administration of phenylephrine may be due an effect of NaF of causing deterioration of the endothelial structure. The decrease in the response to the phenylephrine response in the resveratrol-treated group may be because of the counter-regulatory role of NO in the case of vasodilatation of the intact endothelium without damage to keep the vessels in a physiological condition. Therefore, these findings may be related to a protective effect of resveratrol on the endothelium.

In this study, acetylcholine which causes endothelium-dependent relaxation, caused a concentration-dependent relaxation effect on isolated rat thoracic artery rings. It was observed that the maximum relaxant effect of acetylcholine in both the male and female rats occurred in the groups that received resveratrol. The least relaxation response in both sexes occurred in the groups in which NaF was administered. Acetylcholine causes a vasodilatation effect by an indirect mechanism. Its binding to the muscarinic receptor leads to the release of NO synthesized by eNOS. The NO produced rapidly passes into the vascular smooth muscle cell and then activates guanylate cyclase which results in increased cGMP and relaxation. The endothelial injury caused by NaF may explain the decrease in the endothelium-dependent relaxation response of the acetylcholine in NaF-treated groups. The resveratrol enhanced endothelial-dependent relaxation responses of acetylcholine found in our study are in accordance with the literature.[31,41]

In our study, sodium nitroprusside, which causes relaxation with a direct effect on vascular smooth muscle, caused a contraction-dependent relaxation effect in isolated rat thoracic arteries precontracted with Phe. The maximum relaxation effect obtained with SNP was found in the male rats in the Res+NaF group while for the female rats it occurred in the control group. The minimum relaxation response occurred in the control group of male rats while for the female rats, the minimum relaxation response was detected in the resveratrol group. The relaxation responses obtained by with 10^{-5} M SNP in the groups whose treatment included NaF (the NaF and the Res+NaF

groups) were not less to those obtained in the other study groups in the male rats and for the female rats the difference between the maximum relaxation response in the control group of 112.76%. and that in the Res+NaF group, of 110.06% was small. This suggests that the NaF- and NO-induced guanylate cyclase interaction, and consequently cGMP-dependent mechanisms in the vasculature, had not deteriorated. While the acetylcholine response inducing endothelial NO release was decreased, the NO releasing SNP response was not impaired. This finding suggests that endothelial dysfunction with chronic fluorine exposure is more likely at the level of endothelial NO release.

CONCLUSIONS

In conclusion, the effect of NaF and resveratrol on blood pressure and the contraction-relaxation responses in thoracic aortic rings in male and female rats was investigated. The findings from our study show that resveratrol has a protective efficacy against the increased blood pressure and possible endothelial damage caused by NaF. Our next goal is to highlight the molecular mechanisms of the deleterious effects of fluorosis on veins and the possible protective effects of resveratrol against fluorosis.

CONFLICT OF INTEREST DECLARATION

The authors declare that they have no conflicts of interest concerning this article.

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