EFFECT OF CHRONIC EXPOSURE TO SODIUM FLUORIDE AND 7,12-DIMETHYLBENZ[A]ANTHRACENE ON SOME BLOOD PARAMETERS AND HEPATIC, RENAL, AND CARDIAC HISTOPATHOLOGY IN RATS

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ABSTRACT: This study aimed to investigate the effects of both sodium fluoride (NaF) and 7,12-dimethylbenz[a]anthracene (DMBA), both separately and in combination, on some blood parameters and hepatic, renal, and cardiac histopathology in rats. Forty male Wistar albino rats, weighing 250–300 g, were randomly divided into one control and three experimental groups (i) a NaF group who received 15 ppm of NaF in their drinking water for 90 days, (ii) a DMBA group who received 10 mg DMBA/kg body weight/po/ weekly for 90 days, and (iii) a NaF+DMBA group who received 15 ppm NaF in their drinking water plus 10 mg DMBA/kg bw/po/weekly for 90 days. The animals in the groups were sacrificed at the end of the 90 days. The AST, ALT, LDH, CK, creatinine, troponin I, and MDA levels increased in the NaF, DMBA, and NaF+DMBA groups compared to the control group, while the WBC, K, Na, Cl, urea, SOD, GSH-Px, CAT, and GSH values showed a statistically significant decrease ($p<0.05$). In addition, the CK-MB significantly increased in the DMBA and NaF+DMBA groups compared to the control group ($p<0.05$). The histological structure of the liver, kidney, and heart tissues in the control group was normal. In the NaF and DMBA groups, degenerative and necrotic changes were detected. In the NaF+DMBA group: (i) the liver exhibited hydropic degeneration and coagulation necrosis in hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions, and mononuclear cell infiltration in the portal region; (ii) the kidneys displayed congestion in the glomerulus and interstitial vessels, interstitial nephritis, diffuse hydropic degeneration, and coagulation necrosis in the tubule epithelium; (iii) the heart showed myocardial hyperemia, severe mononuclear cell infiltration in interstitial tissue, hyaline degeneration, and Zenker's necrosis in myocardium As a result of these blood and oxidative stress parameters and histopathological findings, it was determined that NaF, DMBA, and NaF+DMBA induce toxicity in the liver, kidney, and heart tissues and thus play an important role in the physiopathology of toxicity.

Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); Blood parameters; Chronic fluorosis; Histopathological findings; Rat.

INTRODUCTION

Fluorosis occurs acutely and chronically in people and animals.[1,2] High concentrations of the fluoride ion (F) are noxious to the environment, affecting the health of humans and animals.[3] Chronic fluorosis can result in great economic loss in animal production. Fluoride produces deleterious effects on the skeleton, teeth, and soft tissues.[4,5] These toxic effects interfere with the mineralization process and cause defects that are generally irreversible. Volcanic regions are usually rich in fluoride, and so chronic fluorosis is often present in such areas.[5,6]

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Today, industrial development has made human life considerably easier, but has also brought many environmental problems with it. Industrial pollutants threaten health through their appearance in air, water, soil, and food. Polycyclic aromatic hydrocarbons (PAHs) are produced by incomplete combustion of fossil fuels in internal combustion engines, along with coke production, residential heating, and incineration, as well as with natural events such as forest fires and volcanoes.[7,8] Once PAHs enter the atmosphere, they are washed out by rainwater. The literature mentions that PAHs accumulate naturally in many regions of the world, such as high European mountains.[9,10] Therefore, atmospheric transport and deposition of PAHs are widely prevalent in the ecosystem. PAH and its derivatives, such as 7,12-dimethylbenz[a]anthracene (DMBA), were amongst the earliest atmospheric pollutants to be identified as toxic and carcinogenic to people and animals.[7-10]

Volcanic eruptions contribute a significant amount of PAHs into the atmosphere. Residents of volcanic regions are therefore exposed to naturally-occurring fluorocarbons and toxic human-made PAH derivatives as well.[7-10]

In reviewing the literature, no investigations were found which examined the toxic effects on blood parameters and histopathology in rats exposed to fluorocarbons and PAH derivatives, either individually or together. Therefore, this study aimed to investigate experimentally, in rats, the effects on blood parameters and hepatic, renal, and cardiac histopathology of fluoride and the PAH derivative, 7,12-dimethylbenz[a]anthracene, both separately and in combination.

MATERIALS AND METHODS

In this study, 40 male Wistar albino rats, weighing 250–300 g, were randomly divided four groups comprising one control and three experimental groups (n=10 per group). Animals were housed in a well-ventilated and air-conditioned area provided with an independently adjustable light–dark cycle (12 hr light/12 hr dark cycle) and a temperature regulation system. 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.

Groups were formed in the following way: (i) the 1st group rats (control group) were set as control, (ii) the 2nd group rats (NaF group) received 15 ppm fluoride in the form of sodium fluoride (NaF) in their drinking water for 90 days,[11] (iii) the 3rd group rats (DMBA group) received DMBA (10 mg/kg body weight/po/weekly) for 90 days,[12] and (iv) the 4th group rats (NaF+DMBA group) received 15 ppm NaF in their drinking water and DMBA (10 mg/kg bw/po/weekly) for 90 days. On the 90th day of the experiment, intracardiac blood samples were taken under anesthesia with thiopental sodium (20 mg/kg bw) and rats sacrificed by cervical dislocation method. Blood parameters were determined in whole blood by the using rat mode of veterinary practice with a blood cell counter (Abocus Junior Vet-5, Austria). Measurements of biochemical parameters were made with a Modular PP auto-analyzer (Mindray BS800, China). Preparations prepared for histopathological examination of liver, kidney and heart tissues were stained with Hematoxylin-Eosin (H&E) and examined with light microscope (Leica DM 1000). The histopathologic findings was scored as negative (–), slight (+), moderate (++), or severe (+++).

The experimental protocol was approved by the Committee on the Ethics of Animal Experiments at Yuzuncu Yil University (Permit Number: 2015/06-01).

Statistical Analysis of blood parameters were presented as mean±standard deviation (mean±SD). SPSS version 20 was used for statistical analysis. ANOVA and DUNCAN tests were used for comparison between groups.

RESULTS

The results of the hematological parameters and histopathological findings for the groups are presented in Tables 1–5.

Table 1. Some hematological parameters in the groups (NaF=sodium fluoride, DMBA=dimethylbenz[a]anthracene, values are mean±SD; n=10)

Parameter	Control group	NaF group	DMBA group	NaF+DMBA group
WBC ($10^3/mm^3$)	8.23±1.35^a	7.67±1.62^b	5.37±1.21^c	4.29±0.87^d
Lymphocytes (%)	75.28±7.45^a	65.87±8.37^a	71.61±8.34^a	74.06±8.32^a
Monocytes (%)	4.72±1.34^a	5.20±1.32^a	5.11±1.15^a	2.57±1.76^b
Neutrophils (%)	21.32±7.13^a	28.94±7.87^a	25.00±6.91^a	23.37±9.27^a
Platelets ($10^5/mm^3$)	430.00±65.11^a	443.76±62.25^a	470.71±71.84^a	477.66±70.42^a
AST (U/L)	64.16±3.88^a	73.00±3.56^b	77.17±4.61^{bc}	83.16±4.66^c
ALT(U/L)	22.16±3.49^a	32.00±4.14^b	34.33±5.12^b	38.66±3.01^b
LDH (U/L)	410.00±41.25^a	502.33±44.91^b	502.83±39.53^b	539.66±57.84^b
CK (U/L)	312.60±36.14^a	410.68±46.09^b	477.50±33.72^{bc}	511.33±50.80^c
CK-MB (U/L)	421.55±51.01^a	478.35±24.13^a	494.33±59.10^{ab}	549.83±45.03^b
K (mmol/L)	6.93±0.34^a	6.28±0.22^b	6.15±0.36^b	5.8±0.48^c
Na (mmol/L)	144.33±8.26^a	126.00±6.77^b	119.33±8.57^b	118.50±8.87^b
Cl (mmol/L)	110.16±5.23^a	93.66±5.85^b	92.33±4.96^b	90.66±5.08^b
Urea (mg/dL)	61.16±5.66^a	42.16±4.21^b	41.66±4.88^b	39.66±5.98^b
Creatinine (mg/dL)	0.36±0.04^a	0.47±0.04^b	0.49±0.05^b	0.58±0.07^b
Troponin I (µg/L)	0.34±0.04^a	0.44±0.05^b	0.49±0.06^b	0.53±0.09^b

The differences among the different letters in the same line were significant:
a,b: $p<0.05$; a,c: $p<0.01$

Table 2. The levels of MDA, SOD, GSH-Px, CAT and GSH in the groups (NaF=sodium fluoride, DMBA=dimethylbenz[a]anthracene, pt=protein, values are mean±SD; n=10)

Tissue sample	Oxidative stress parameters	Control group	NaF group	DMBA group	NaF+DMBA group
Liver	MDA (nmol/mg pt)	0.96 ±0.071[a]	1.03 ±0.081[b]	1.39 ±0.082[c]	1.63 ±0.098[d]
	SOD (IU/mg pt)	561.05 ±18.46[a]	465.19 ±19.53[b]	472.58 ±19.75[b]	418.12 ±19.67[c]
	GSH-Px (IU/mg pt)	2.18 ±0.096[a]	1.78 ±0.10 [b]	1.74 ±0.099 [b]	1.58 ±0.079[b]
	CAT (IU/mg pt)	29.65 ±1.18 [a]	25.68 ±1.11 [b]	22.96 ±1.17[c]	20.96 ±0.98[c]
	GSH (µmol/mg pt)	0.0276 ±0.0012[a]	0.0219 ±0.00099[b]	0.0222 ±0.0011[b]	0.0189 ±0.0014[c]
Kidney	MDA (nmol/mg pt)	1.04 ±0.089[a]	1.26 ±0.086[b]	1.47 ±0.107[c]	1.56 ±0.102[c]
	SOD (IU/mg pt)	396.94 ±8.79[a]	377.22 ±6.94[b]	373.96 ±9.11[b]	346.08 ±8.69[c]
	GSH-Px (IU/mg pt)	2.09 ±0.096[a]	1.80 ±0.095[b]	1.63 ±0.099[c]	1.39 ±0.11[d]
	CAT (IU/mg pt)	18.96 ±0.56[a]	16.95 ±0.52[b]	16.78 ±0.92[b]	14.56 ±0.75[c]
	GSH (µmol/mg pt)	0.0218 ± 0.0011[a]	0.0183 ±0.00094[b]	0.0168 ±0.00079[b]	0.0152 ±0.00052[c]
Heart	MDA (nmol/mg pt)	1.01 ±0.065[a]	1.26 ±0.076[b]	1.48 ±0.14[c]	1.61 ±0.10[c]
	SOD (IU/mg pt)	284.11 ±12.25[a]	227.09 ±10.39[b]	226.38 ±12.10[b]	212.65 ±12.78[b]
	GSH-Px (IU/mg pt)	1.74 ±0.083[a]	1.46 ±0.099[b]	1.39 ±0.12[b]	1.28 ±0.094[b]
	CAT (IU/mg pt)	8.69 ±0.39[a]	7.66 ±0.27[b]	6.59 ±0.40[c]	6.14 ±0.29[c]
	GSH (µmol/mg pt)	0.0199 ±0.0010[a]	0.0176 ±0.0010[b]	0.0163 ±0.0013[b]	0.0135 ±0.00096[c]

The differences among the different letters in the same line were significant:
a,b: $p<0.05$; a,c: $p<0.01$

Table 3. Histopathological evaluation of liver tissue. (NaF=sodium fluoride, DMBA=dimethylbenz[a]anthracene, rating of histopathologic findings: negative = –, slight = +, moderate = ++, severe = +++)

Histopathological finding	Control group	NaF group	DMBA group	NaF+DMBA group
Hidropic degeneration in hepatocytes	–	++	+	+++
Coagulation necrosis in hepatocytes	–	–	–	+++
Hyperemia	–	+	+	+++
Mononuclear cell infiltration	–	+	++	+++

Table 4. Histopathological evaluation of kidney tissue. (NaF=sodium fluoride, DMBA=dimethylbenz[a]anthracene, rating of histopathologic findings: negative = –, slight = +, moderate = ++, severe = +++)

Histopathological finding	Control group	NaF group	DMBA group	NaF+DMBA group
Hidropic degeneration in tubule epithelium	–	++	+	+++
Coagulation necrosis in tubule epithelium	–	+	–	+++
Intertubular and glomerular hyperemia	–	–	–	+++
Mononuclear cell infiltration	–	+	++	+++
Dilation in Bowman capsule	–	+	–	++

Table 5. Histopathological evaluation of heart tissue. (NaF=sodium fluoride, DMBA=dimethylbenz[a]anthracene, rating of histopathologic findings: negative = –, slight = +, moderate = ++, severe = +++)

Histopathological finding	Control group	NaF group	DMBA group	NaF+DMBA group
Hyperthermia in interstitial capillaries	–	++	+	+++
Hyaline degeneration in muscle fibers	–	++	–	+++
Zenker's necrosis in muscle fibers	–	+	–	+++
Mononuclear cell infiltration	–	++	+	+++

The values in the control groups were inside the range of changes in the values of healthy rats. As seen in Table 1, AST, ALT, LDH, CK, creatinine, and troponin I levels increased in the NaF, DMBA, and NaF+DMBA groups compared to the control group, while the white blood cell (WBC), K, Na, Cl, and urea values decreased by a statistically significant amount ($p<0.05$). In addition, CK-MB showed a statistically significant increase in the DMBA and NaF+DMBA groups compared to the control group ($p<0.05$). It was determined that lymphocyte (%), neutrophil (%), and platelet numbers did not change significantly in the groups with their values being closer to the mean values and within the physiological variation limits ($p>0.05$).

The state of free radicals and antioxidants is given in Table 2. As seen in Table 2, the levels of MDA increased in liver, kidney, and heart tissues in the NaF, DMBA, and NaF+DMBA groups compared to the control group, while SOD, GSH-Px, CAT, and GSH values decreased significantly ($p<0.05$).

Histopathological findings: The control group showed normal histological structure in the liver tissue (Figure 1A). The histopathological findings detected in the livers of the NaF group were degeneration in the acinar regions, slight cholangiohepatitis in the portal region, dilatation in the sinusoids, and congestion in the sinusoidal spaces and veins in the portal region (Figure 1B). In the DMBA group, the livers exhibited dilatation of the sinusoids, cholangiohepatitis in the portal region, and congestion in the sinusoidal and portal regions (Figure 1C). In the livers of the NaF+DMBA group the histopathological findings observed were hydropic degeneration and coagulation necrosis in the hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions, and mononuclear cell infiltration in the portal region (Figure 1D). Histopathologic findings in the livers are summarized in Table 3.

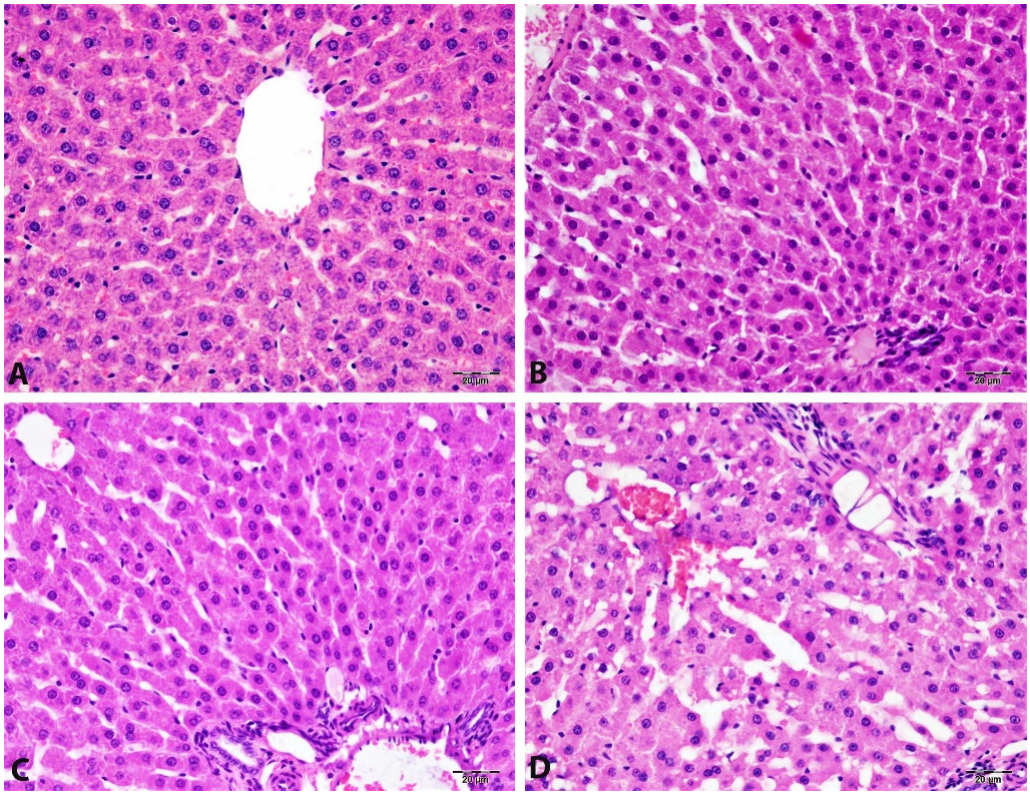

Figure 1. A: Control group showing liver tissue with a normal histological structure; B: NaF group showing dilation in sinusoids, mild mononuclear cell infiltration in the portal region, and hydropic degeneration in hepatocytes; C: DMBA group showing colangiohepatitis in portal region, mild dilation in sinusoids, hyperemia, and hydropic degeneration in hepatocytes. and D: NaF+DMBA group showing severe dilatation in sinusoids, hydropic degeneration in hepatocytes and coagulation necrosis, and colangiohepatitis. (Staining = H&E, bar = 20 μm).

The control group exhibited normal histological structure in the kidney tissues (Figure 2A). The histopathological findings in the kidneys of the NaF group were congestion in the glomerulus and intertubular spaces, mild interstitial nephritis, dilatation in the bowman capsule, and degeneration and a few necroses in the tubular epithelium (Figure 2B). In the kidneys of the DMBA group the histopathological findings observed were slight interstitial nephritis, degeneration in the tubular epithelium, and congestion in the glomerulus and intertubular spaces (Figure 2C). The kidneys of the NaF+DMBA group revealed congestion in the glomerulus and interstitial vessels, interstitial nephritis, diffuse hydropic degeneration, and coagulation necrosis in the tubular epithelium (Figure 2D). Histopathologic findings of the kidneys are summarized in Table 4.

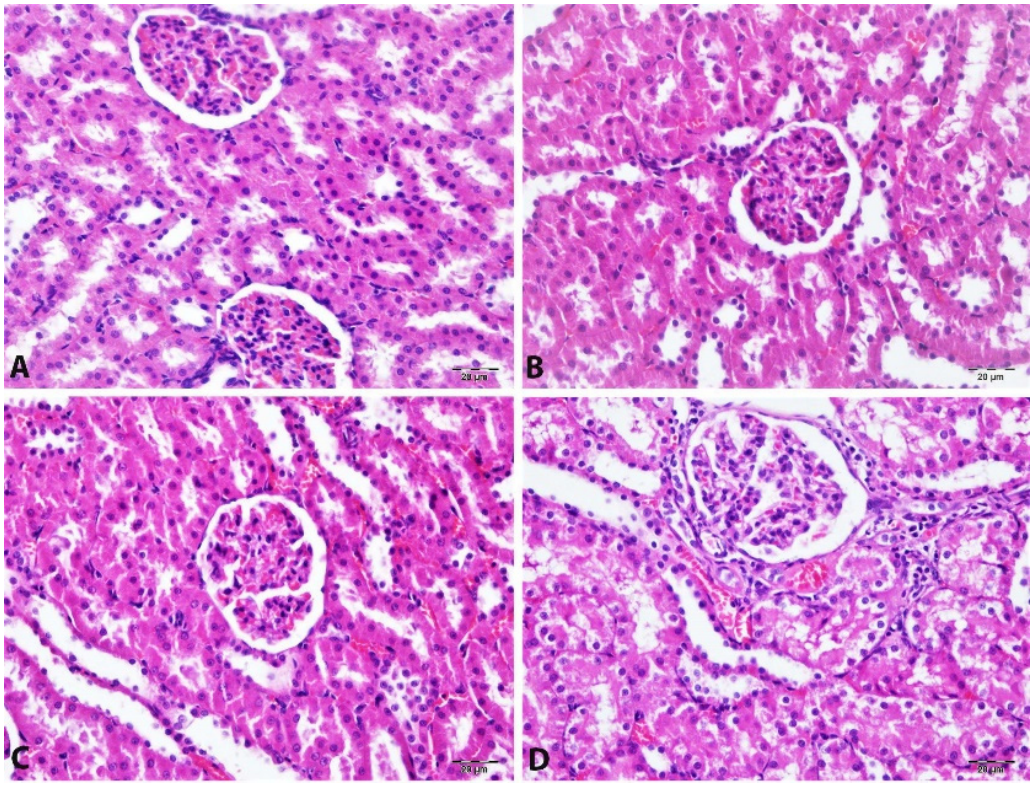

Figure 2. A: Control group showing kidney tissue with a normal histological structure; B: NaF group showing mild dilatation in Bowman capsule, hydropic degeneration in tubular epithelium, and hyperemia in glomerular and intertubular intervals; C: DMBA group showing mild dilatation in Bowman capsule and some tubules, hyperemia in vessels in glomerular and intertubular spaces, and mild hydropic degeneration in tubular epithelium; D: NaF+DMBA group showing dilatation in Bowman capsule and some tubules, diffuse hydropic degeneration and coagulation necrosis in tubular epithelium, and mononuclear cell infiltration in interstitial tissue. (Staining = H&E, bar = 20 μm).

The control group showed normal histological structure in the heart tissues (Figure 3A). In the heart tissues of the NaF group, hyperemia in the interstitial vessels was detected along with hyaline degeneration and Zenker's necrosis in the myocardium (Figure 3B). In the heart tissues of the DMBA group a small number of mononuclear cell infiltrations, were found together with mild hyperemia at the myocardial interstitial intervals and in the capillaries (Figure 3C). In the heart tissues of the NaF+DMBA group, myocardial hyperemia, severe mononuclear cell infiltration in interstitial tissue, as well as hyaline degeneration and Zenker's necrosis in the myocardium were detected (Figure 3D). Histopathologic findings of the heart tissues are summarized in Table 5.

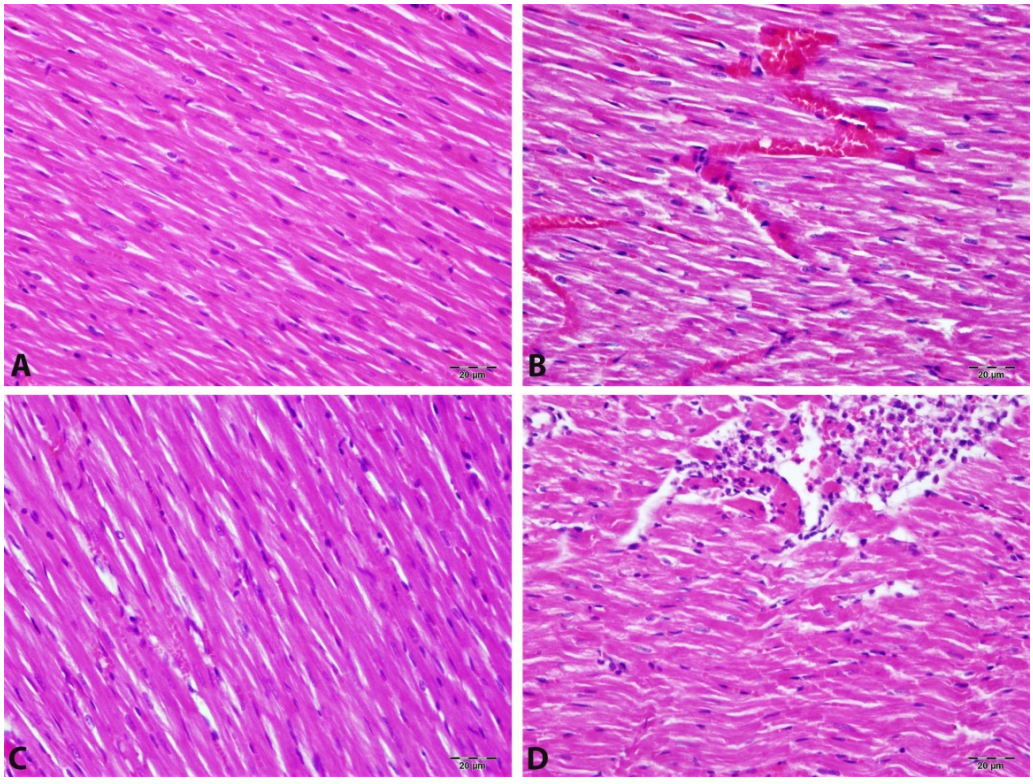

Figure 3. A: Control group showing heart tissue with a normal histological structure; B: NaF group showing hyperemia in interstitial vessels, and hyaline degeneration and Zenker's necrosis in muscle fibers; C: DMBA group showing hyperemia and mild mononuclear cell infiltration in interstitial intervals; D: NaF+DMBA group showing hyperemia in the interstitial vessels of myocardium, severe mononuclear cell infiltration in interstitial tissue, and hyaline degeneration and Zenker's necrosis in muscle fibers. (Staining = H&E, bar = 20 μm).

DISCUSSION

In recent years, researchers have realized that fluoride accumulates not only in bones and teeth but to a lesser extent in soft tissues, especially in the cardiovascular system. Fluoride can rapidly cross the cell membrane and be distributed in teeth, bones, myocardium, liver, skin, and erythrocytes.[13-16] High concentrations of fluoride are noxious for the environment, affecting the health of humans and animals.[4,15-18] Volcanic sites are rich in fluoride and PAHs, leading, in such regions, to chronic fluorosis on one hand and PAH toxicity on the other.[7-9,19-21]

PAHs, which arise from incomplete burning of organic compounds, are organically structured compounds which have toxic and carcinogenic effects. PAHs are taken into the body with air, water, food, and smoke, and they cause mutations in DNA. Over 100 PAH compounds have been found in nature. Of these, 16 have been given top priority because their carcinogenic and toxic effects are higher.[7-10]

Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition. In the present study, the effects on blood parameters and hepatic, renal, and cardiac histopathology of fluoride and the

PAH derivative, 7,12-dimethylbenz[a]anthracene, both separately and in combination, were studied (Tables 1–5 and Figures 1–3).

As seen in Table 1, AST, ALT, LDH, CK, creatinine, and troponin I levels increased in the NaF, DMBA, and NaF+DMBA groups compared to the control group, with a significance of $p<0.05$. In addition, CK-MB increased statistically significantly in the DMBA and NaF+DMBA groups compared to the control group ($p<0.05$). AST and ALP enzymes are believed to reflect liver damage in rats. AST is concentrated in many places such as the liver, heart, muscle, brain, pancreas, kidney, and lung, as well as in the white and red blood cells. In contrast, ALT is only present to a substantial degree in the liver. AST and ALT levels are significantly greater in cases of liver disease, compared to healthy individuals. This finding is consistent with the literature.[12,22,23] In the present study, degeneration and necrosis were seen in hepatocytes caused by the effects of NaF+DMBA toxicity (Figure 1D). Total CK, CK-MB activity, LDH, AST, and cardiac troponin I are important in determining the presence of heart disease.[22,23] In our study, in the NaF+DMBA group, histological examination of the heart muscle revealed myocarditis, hyaline degeneration, and Zenker's necrosis (Figure 3D). These are very important findings. As seen in Figures 1 and 3, increases in these parameters may be due to the effects of fluoride and DMBA toxicity on the liver and heart.

In the present study, WBC, K, Na, Cl, and urea values in the NaF, DMBA, and NaF+DMBA groups decreased significantly compared to the control group ($p<0.05$). The lymphocytes, neutrophils, and platelets did not change significantly in the groups, with their values being closer to the mean values and physiological variation limits ($p>0.05$). In general, the blood parameters of the rats in the control group are consistent with the literature.[12,22,23]

Electrolytes in body fluids ensure the regular functioning of heart, nerve, and muscle. Some electrolytes are building blocks of hard tissues such as bones and teeth. The kidneys play an important role in the balance of electrolyte levels. The blood level of urea, produced in the liver and excreted by the kidney, is regarded as an important criterion in kidney function.[22-24] Reductions in K, Na, Cl, and urea values in the NaF, DMBA, and NaF+DMBA groups may be a consequence of hydropic degeneration and coagulation necrosis in the renal tubule epithelium (Figure 2D).

Reactive oxygen species (ROS) are important as pathological agents for many diseases. Increased oxygen radical production and lipid peroxidation are associated with the pathogenesis of many diseases and the toxic effects of a wide range of compounds.[25,26] Many studies have indicated that excessive fluoride intake may increase lipid peroxidation, while antioxidative enzymes are able to inhibit this in the liver, kidney, heart, ovary, brain, and some muscles.[27-30] Our study indicated that fluoride increased free radical production and inhibited the antioxidative enzymes in the liver, heart, and kidney.

While the MDA level in the NaF group was statistically higher than in the control group ($p<0.05$), the DMBA and NaF+DMBA groups had elevations in all the tissues that were higher than in the other two groups ($p<0.01$) (Tables 1 and 2; Figures 1–3). Similarly, a decline in levels of antioxidant enzymes (SOD, GSH-Px, CAT, and GSH) was found to be greater in the DMBA and NaF+DMBA groups than in the NaF

group. As seen in the histopathologic findings, this may be due to the damaging effects of DMBA and NaF+DMBA on liver, kidney, and heart tissue. These findings are seen to be in agreement with the literature. However, some reports conflict with our findings. Reddy et al.[31] found that lipid peroxide, GSH, and vitamin C levels, as well as SOD, GSH-Px, and CAT activities, caused no change in the red blood cells of fluorotic humans and rabbits. Chlubek et al.[32] reported a decrease in cytoplasmic SOD activity but no change in GSH-Px activity and MDA content. These differences may be due to differences in the species, dose, sensitivity, and age of the animals.[33]

Ersan et al.[11] observed hydropic degeneration and necrosis in hepatocytes in the acinar region, along with hyperemia in the livers of chronic fluorosis rats. In a different study, chronic fluorosis was induced in rats by adding 25 ppm fluoride to the animals' drinking water for 12 weeks resulting in the hepatic changes of degeneration, necrosis, and mononuclear cell infiltration in the portal region.[34] These findings were similar to the livers of the NaF group in our study. In the DMBA-induced liver damage studies, the livers of the rats exhibited hepatocyte necrosis, congestion in the sinusoids, and mononuclear cell infiltration in the portal region.[12,35,36] In our study, the findings in the livers of the DMBA-treated rats were comparable with the literature. In the livers of the rats in the NaF+DMBA group we found sinusoidal dilatation, hyperemia, severe mononuclear cell infiltration in the portal region, and severe degeneration and necrosis in hepatocytes in the periapical region (Table 3 and Figure 1).

In a study in which chronic fluorosis occurred in rats after adding sodium fluoride to their drinking water for 12 weeks, degenerative changes in the cortex and medullary tubular epithelium, together with glomerular hyperemia in kidneys, were observed.[37] In another chronic fluorosis study, there changes observed were dilatation in the distal and proximal tubules, perivascular and peritubular mononuclear cell infiltration in the interstitial space, congestion in the majority of vascular structures, and severe degenerative changes in the tubular epithelium.[38] In our study, the NaF group showed congestion in the veins, intertubular spaces, and glomeruli, along with mild interstitial nephritis, dilation in the Bowman capsule, degeneration of tubule epithelium, and some necrosis in the tubular epithelium. Özdemir et al.[12] reported that mild hydropic degeneration and hyperemia in the vascular structures in the kidneys occurred after DMBA was administered orally by single doses of 75 mg/kg. Our study had similar findings in the DMBA group. In the NaF+DMBA group we observed congestion in glomerulus and interstitial vessels, interstitial nephritis, diffuse degeneration, and coagulation necrosis in the tubular epithelium (Table 4 and Figure 2).

One study reported that heart tissue samples of rats with chronic fluorosis showed myocarditis with cloudy swelling, necrosis, hemorrhage, inflammation, and atherosclerosis.[39] Another experimental fluorosis study reported that examination of the heart revealed congestion in the capillaries, mononuclear cell infiltration and hemorrhage in the myofibrillar interval, severe degeneration of the muscles, and numerous vacuoles in the cytoplasm.[40] In our study, the NaF group revealed hyperemia in the capillary vessels, together with hyaline degeneration and Zenker's necrosis in the heart muscle. In the NaF+DMBA group there we found hyperemia in the capillary vessels, severe mononuclear cell infiltration in the interstitial interval,

and hyaline degeneration and Zenker's necrosis in the heart muscle (Table 5 and Figure 3).

CONCLUSIONS

According to the blood and oxidative stress parameters and histopathological findings of the present study, chronic fluorosis+DMBA toxicity were found to cause excessive production of oxygen free radicals, enhance lipid peroxidation, increase AST, ALT, LDH, CK, CK-MB, creatinine, and cardiac troponin I values, decrease K, Na, Cl, and urea levels, and inhibit antioxidative enzymes. It appears that oxidative stress and damage to liver, heart, and kidney were induced by NaF, DMBA, and NaF+DMBA and thus play an important role in the pathogenesis of toxicity.

Thus, the changes in the blood and oxidative stress parameters and the hepatic, renal, and cardiac histopathology play an important role in the physiopathology of the toxicity induced in the liver, kidney, and heart tissues by NaF, DMBA, and NaF+DMBA.

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