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Exercise Ameliorates Fluoride-induces Cardiac Inflammation in Mice

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ABSTRACT

Purpose: Fluoride exposure adversely affects cardiac morphological structure and function. In contrast, moderate exercise promotes cardiac function and immune homeostasis. However, the alleviating roles of exercises on cardiac inflammation caused by fluorosis remain a huge unknown. Therefore, this study was designed to investigate the effects of moderate exercise on alterations in morphological structure and the levels of inflammatory factors in cardiac tissue in mice exposed to fluoride.

Methods: 48 female mice were randomly divided into four groups: control group, exercise group, fluoride group, and exercise + fluoride group. 6 months later, the morphological examination of myocardium, the CD68 level and the mRNA expressions of NF-κB, IL-1β, IL-6, IL-10 and TNF-α in heart tissue were detected, respectively.

Results: Histological examinations using HE staining and transmission electron microscopy (TEM) revealed that exercise mitigated cardiac morphological injury in mice exposed to fluoride. Immunofluorescence staining indicated an increase in the expression of the macrophage marker CD68 in the F group and a decrease in the exercise + fluoride group, suggesting that exercise alleviated the cardiac inflammatory response elicited by fluoride. Furthermore, exercise significantly reduced the mRNA levels of pro-inflammatory factors, including IL-6, IL-1β, and TNF-α, while elevating the expression of the anti-inflammatory factor IL-10 in mice exposed to fluoride for 6 months.

Conclusions: Findings in this study indicated that moderate physical activities exerted a protective effect on mice by reducing inflammation and enhancing anti-inflammatory responses, potentially informing strategies for preventing fluoride-related cardiotoxicity.

Key-words: *Cardiac inflammation; Exercise; Fluoride; Mouse.*

INTRODUCTION

Epidemiological studies have indicated that endemic fluorosis is the presence of pathological changes in both bone and non-skeletal tissues, including the reproductive, nervous, digestive, and cardiovascular systems.[1-5] Excessive fluoride ingestion has been linked to heart enlargement and electrocardiac changes in humans.[6] Accumulated investigations have gained momentum in illustrating the pathogenesis leading to cardiac dysfunction upon exposure to fluoride.[7-10] Inflammation is a major pathogenic feature of cardiovascular diseases and plays a pivotal role in the mechanism of fluoride-related cardiotoxicity by which fluoride triggers a myocardial inflammatory response by activating NF-κB, which in turn activates inflammatory cytokines, such as interleukins (ILs) and tumor necrosis factor (TNF).[11-13] Increased TNF-α protein expression was observed in fluoride-treated rats,[13] and it was discovered that the mRNA and protein levels of IL-6 and IL-10 showed a marked decrease in these rats.[9] Furthermore, according to protein-protein interaction network analysis, IL-6 and IL-10 play an important role in the cardiac lesions induced by fluoride as an external stimulus.[9]

It is widely accepted that exercise has an anti-inflammatory effect, and part of the benefits associated with post-exercise can also be attributed to these anti-inflammatory properties.[14] Following exercise, there is a noted decrease in the concentration of pro-inflammatory factors and an increase in anti-inflammatory mediators.[15-16] Studies indicated that chronic exercise training enhanced motor ability as well as improved left ventricular diastolic function and stiffness in rats, which correlated with a reduction in circulating inflammatory cytokine levels.[17] Notably, exercise provoked a significant increase in IL-6 and IL-10, exerting a direct anti-inflammatory effect by inhibiting TNF-α and stimulating IL-1RA, thereby limiting IL-1β signaling.[18]

Recent studies from our lab revealed that exercise ameliorates intestinal damage and microbial disturbances,[19] as well as anxiety- and depression-like behaviors in mice exposed to fluoride.[20] However, the palliative effect of exercise on cardiac inflammation caused by fluorosis remains a huge unknown. Therefore, this study was designed to investigate the alterations in morphological structure and the levels of inflammatory factors in cardiac tissue resulting from fluoride exposure and/or exercise.

MATERIAL AND METHODS

Animals and treatments: Four-week-old SPF-grade female ICR mice (n=48) were obtained from the Experimental Animal Center of Shanxi Medical University (Taiyuan, China) and maintained on standard diets. The mice were housed in a spacious and clean environment, with access to adequate standard diets and clean water. The 48 female mice were randomly assigned to four groups of 12 each: control group (C), fluoride group (F), exercise group (E), and exercise + fluoride group (EF). Mice in groups C and E received distilled water, while those in groups F and EF were administered 100 mg/L sodium fluoride (NaF). All animal use and experimental procedures were conducted in accordance with the guidelines set forth by the Experimental Animal Ethics Committee of Shanxi Agricultural University (Jinzhong China).

Endurance training program： Previous studies have indicated that an intensity within the range of 30%-45% VO_{2max} is considered the minimum intensity for improving cardiorespiratory function, which can yield positive fitness effects.[21] In this experiment, referencing 40% of the maximum aerobic speed in mice,[22,23] the exercise regimen was set at 12 m/min, 30 min/day, 5 days/week for training. Mice in the E and EF groups engaged in endurance training 5 days per week for a duration of 6 months, maintaining a work rate of approximately 40% of VO_{2max}. The first stage of the exercise regimen consisted of 2 minutes of exercise at 5 m/min, followed by 26 minutes at 10 m/min, then 2 minutes at 5 m/min again, culminating in continuous training for a period of 3 weeks. In the second stage, the protocol included 2 minutes at 5 m/min, followed by 26 minutes at 12 m/min, again concluding with 2 minutes at 5 m/min, and extending continuous training for 5 months.

Morphological observation of the heart tissue: Sections of heart tissue were examined using conventional light microscopy with HE staining. Three heart samples from each group were fixed in 4% paraformaldehyde solution for 36 hours, dehydrated in xylene and a graded series of ethanol, and subsequently embedded in paraffin. Continuous sections (5 μm) were cut using a Leica RM2265 microtome (Germany) and stained with HE.

TEM observation: The ultrastructure of heart tissue was examined using TEM. Approximately 1 mm × 1 mm × 1 mm samples from fresh heart tissues of three mice in each group were washed with saline and subsequently fixed in 1% glutaraldehyde in 0.1 M phosphate buffer (PB) at pH 7.4 at room temperature. After a fixation period of 2 hours, the samples were further fixed in 2.5% osmium tetroxide in 0.1 M PB. The specimens were then sectioned to a thickness of 70 nm and observed by TEM (JEM 1011).

Activities of the aspartate aminotransferase (AST) and the creatine kinase (CK): The activities of AST and CK in heart tissue were assayed using enzyme kits from Jiancheng Biochemistry Institute, Nanjing, Jiangsu, China. All procedures were conducted in accordance with the provided instructions.

Immunofluorescence staining of CD68: Heart tissue samples were fixed in a 4% paraformaldehyde solution for 36 hours and subsequently dehydrated using xylene and a series of graded ethanol solutions. Following embedding in paraffin, the samples were sectioned at a thickness of 4 μm and placed onto slides. The sections were washed, rinsed, and subjected to boiling in sodium citrate buffer for 15 minutes to facilitate antigen retrieval. After rinsing in phosphate-buffered saline (PBS), the sections were blocked with 5% bovine serum albumin (BSA) for 2 hours at room temperature. Subsequently, the sections were incubated with the primary antibody overnight at 4°C. After washing three times with PBST, the sections were incubated with a CY3-conjugated secondary antibody (Proteintech Group, Wuhan, Hubei, China) for 2 hours at room temperature, followed by three additional washes with PBST. Finally, the sections were

sealed with a mounting medium containing 4,6-diamidino-2-phenylindole (DAPI, Solarbio Science & Technology Co., Beijing, China). Images were acquired immediately using a fluorescence microscope (BX53F, Olympus, Japan).

QRT-PCR analysis: Total RNA was extracted from heart tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) for quantitative reverse transcription polymerase chain reaction (QRT-PCR). The RNA concentration and quality were assessed by measuring the A260/280 and A260/230 ratios using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, Delaware, USA). The RNA samples were then reverse-transcribed with the PrimeScript RT Master Mix kit (Takara Bio, Japan). Primer sequences along with their corresponding PCR product sizes are presented in Table 1. The thermocycling conditions were set as follows: 95 °C for 5 minutes, followed by 40 PCR cycles of 95 °C for 15 seconds, 60 °C for 30 seconds, and 72 °C for 6 seconds. Subsequently, the data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis: Statistical analyses were conducted using GraphPad Prism 5 software, and the data are presented as the mean±SEM. Statistical differences were assessed using one-way analysis of variance (ANOVA). p-values of < 0.05 and < 0.01 were considered statistically significant.

Table 1. Primer sequences for QRT-PCR

Gene name	Primer sequence (5'→3')	Product size (bp)	GenBank number
GAPDH	F: ACTCTACCCACGGCAAGTTC R: TACTCAGCACCAGCATCACC	104	NM_031144.5
IL-1β	F: TCAGCACCTCACAAGCAGAG R: TTCTTGTGACCCTGAGCGAC	230	NM_008360.2
TNF-α	F: TGTCCCTTTCACTCACTGGC R: TCTTCTGCCAGTTCCACGTC	98	NM_013693.3
IL-6	F: TTGGGACTGATGCTGGTGAC R: TTGGGACTGATGCTGGTGAC	94	NM_031168.2
IL-10	F: TGCTGCCTGCTCTTACTGAC R: TCTAGGAGCATGTGGCTCTG	107	NM_010548.2
NF-κB	F:GTAACAGCAGGACCCAAGGA R: AGCCCCTAA TACACGCCTCT	121	NM_008689

RESULTS

Morphologic changes in hearts of mice: The morphological changes in the heart tissues of mice from each group were evaluated using a 40x microscope following HE staining of paraffin sections (Figure 1). The control group exhibited normal myocardial cells, characterized by orderly arranged myofilaments, distinct transverse lines, and uniform staining (Figure 1C). In group E, the arrangement of myofilaments appeared more compact and layered (Figure 1E). Conversely, cardiomyocytes in group F demonstrated irregular sequences, accompanied by signs of tearing (Figure 1F). In comparison to group F, the arrangement of myocardial fibers in group EF was more orderly, with clearly visible nuclei and no evidence of nuclear aggregation (Figure 1EF).

Alterations in ultrastructure of mice heart: Ultrastructural changes in the hearts of mice treated with fluoride and/or exercise were observed using TEM (Figure 2). The myofilaments displayed complete and well-regulated structures, with clearly visible Z-lines, and the mitochondria maintained their structural integrity, exhibiting distinct mitochondrial ridges in C (Figure 2A) and E (Figure 2B) groups. In contrast, Figure 2C illustrates myocardial fiber breakage, intercalated disk disruptions, Z-line interruptions, and slight mitochondrial vacuolization along with mitochondrial ridge dissolution in the F group. Notably, the ultrastructure of cardiac tissue in the EF group showed significant improvement compared to that in the F group (Figure 2D). The structure of myocardial fibers, intercalated disks, and mitochondria remained

intact, with myocardial fibers arranged neatly, although a slight degree of vacuolation was still present.

Activities of AST and CK in mice heart: The activities of heart-related enzymes AST and CK are shown in Fig. 3. The activities of AST and CK in F were significantly higher compared with C ($p < 0.01$; $p < 0.05$).

Detection of the macrophages marker CD68 in the mice heart: Figure 4 displays the immunofluorescence staining of CD68 in each group. Compared to the control group, the expression of CD68 was significantly increased in group F ($p < 0.05$), as indicated by the presence of red granules in the image. Conversely, the expression of CD68 was significantly decreased in group EF ($p < 0.05$).

mRNA expressions of NF-κB, IL-1β, IL-6, IL-10, and TNF-α: The mRNA levels of NF-κB, IL-1β, IL-6, IL-10, and TNF-α in heart tissue from mice are presented in Figure 5. Compared to the control group, the mRNA levels of NF-κB, IL-1β, IL-6, and TNF-α were significantly elevated in group F ($p < 0.05$; $p < 0.01$). In contrast, when comparing group EF to group F, the mRNA levels of IL-1β, IL-6, and TNF-α were significantly reduced, whereas the mRNA level of IL-10 was significantly increased in group EF ($p < 0.05$; $p < 0.01$).

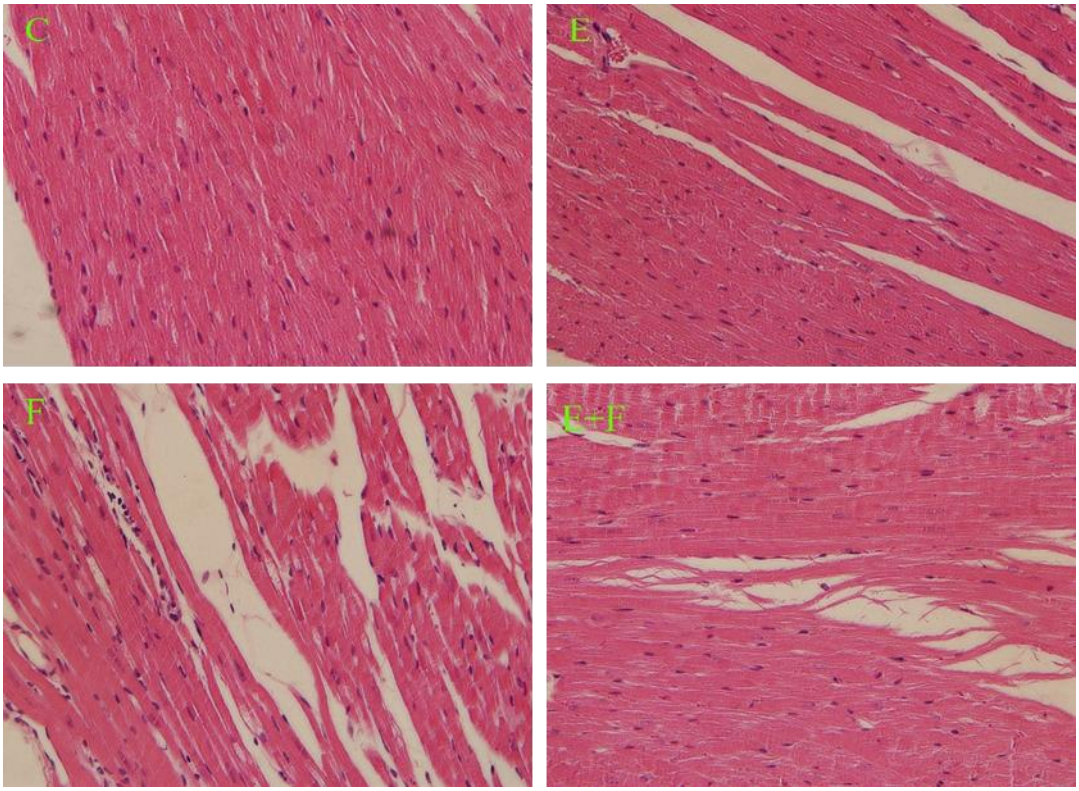

Figure 1. Histopathological photographs of the cardiac muscle in mice in control (C), exercise (E), fluoride (F), and exercise + fluoride (EF) groups. The magnification is ×400.

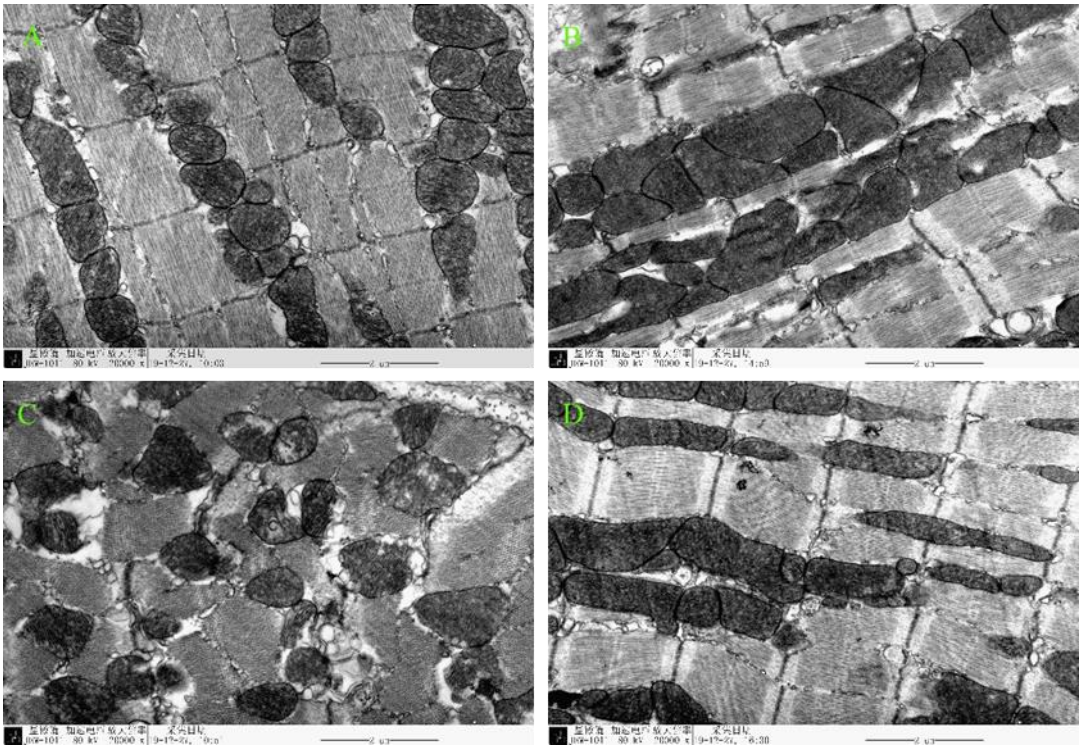

Figure 2. Cardiac muscle fibers of mice in control (C), exercise (E), fluoride (F), and exercise + fluoride (EF) groups. (20000×)

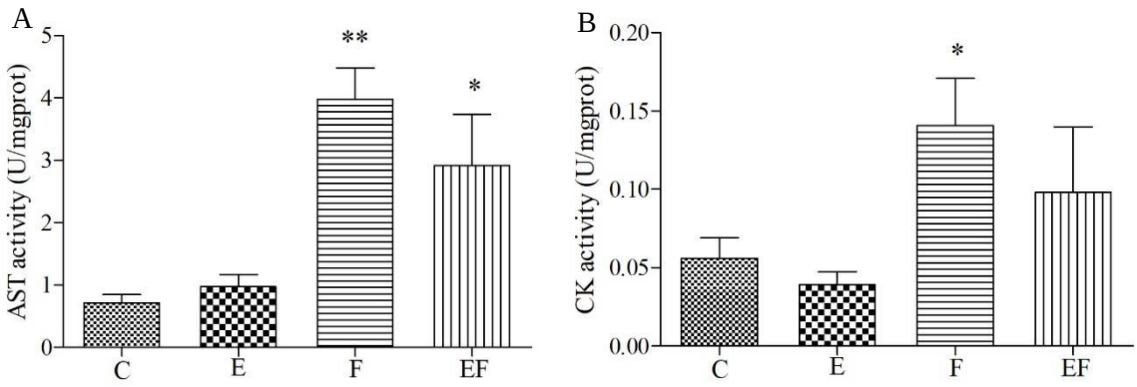

Figure 3. The activities of heart-related enzymes AST and CK.

"*" or "**" indicates a statistically significant difference compared to group C ($P<0.05$; $P<0.01$).

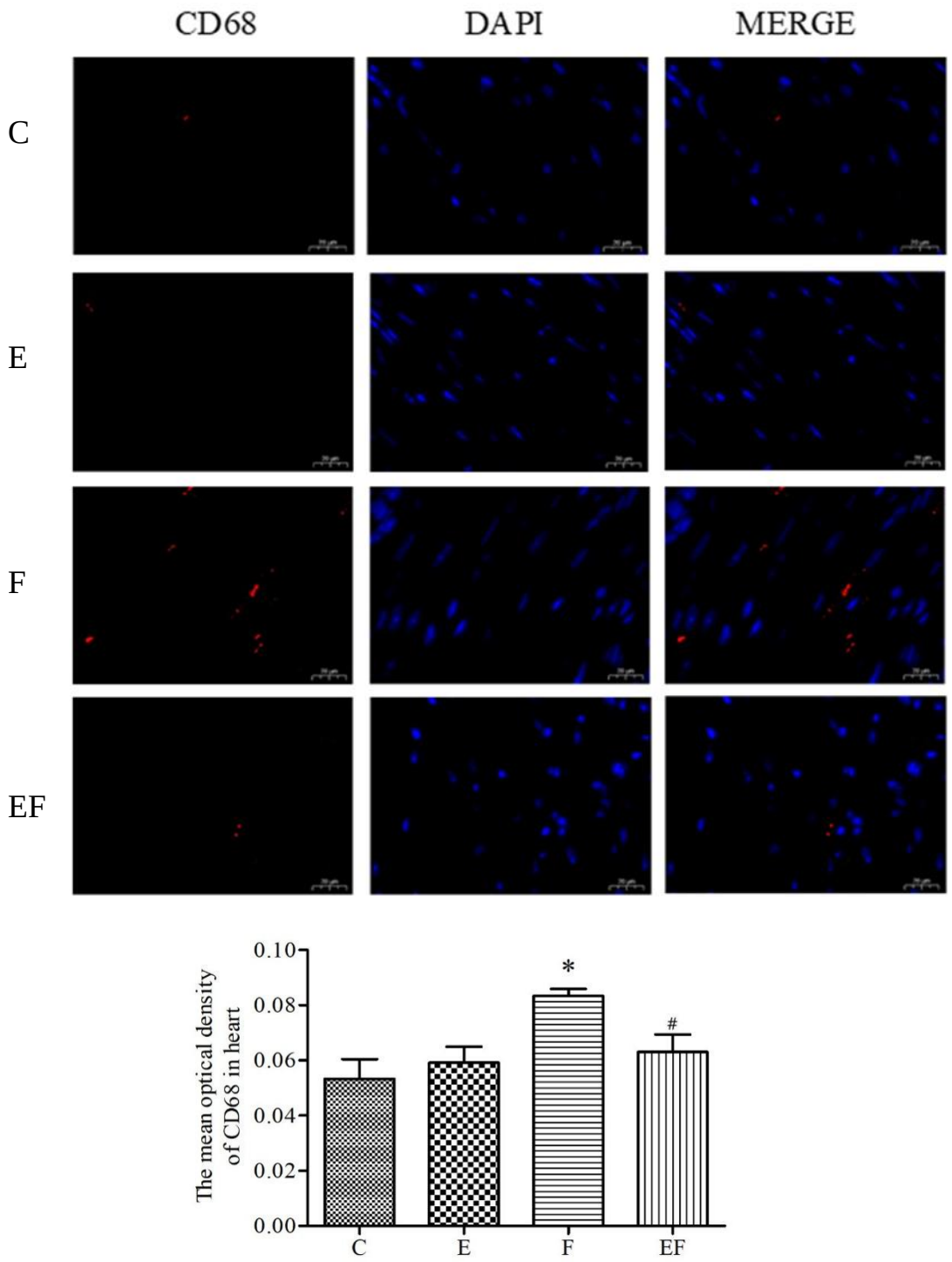

Figure 4. Immunofluorescence labeling and the average optical density of macrophage CD68 in mice control (C), exercise (E), fluoride (F), and exercise + fluoride (EF) groups.

"*" indicates a statistically significant difference compared to group C ($P < 0.05$).
"#" indicates a statistically significant difference compared to group F ($P<0.05$).

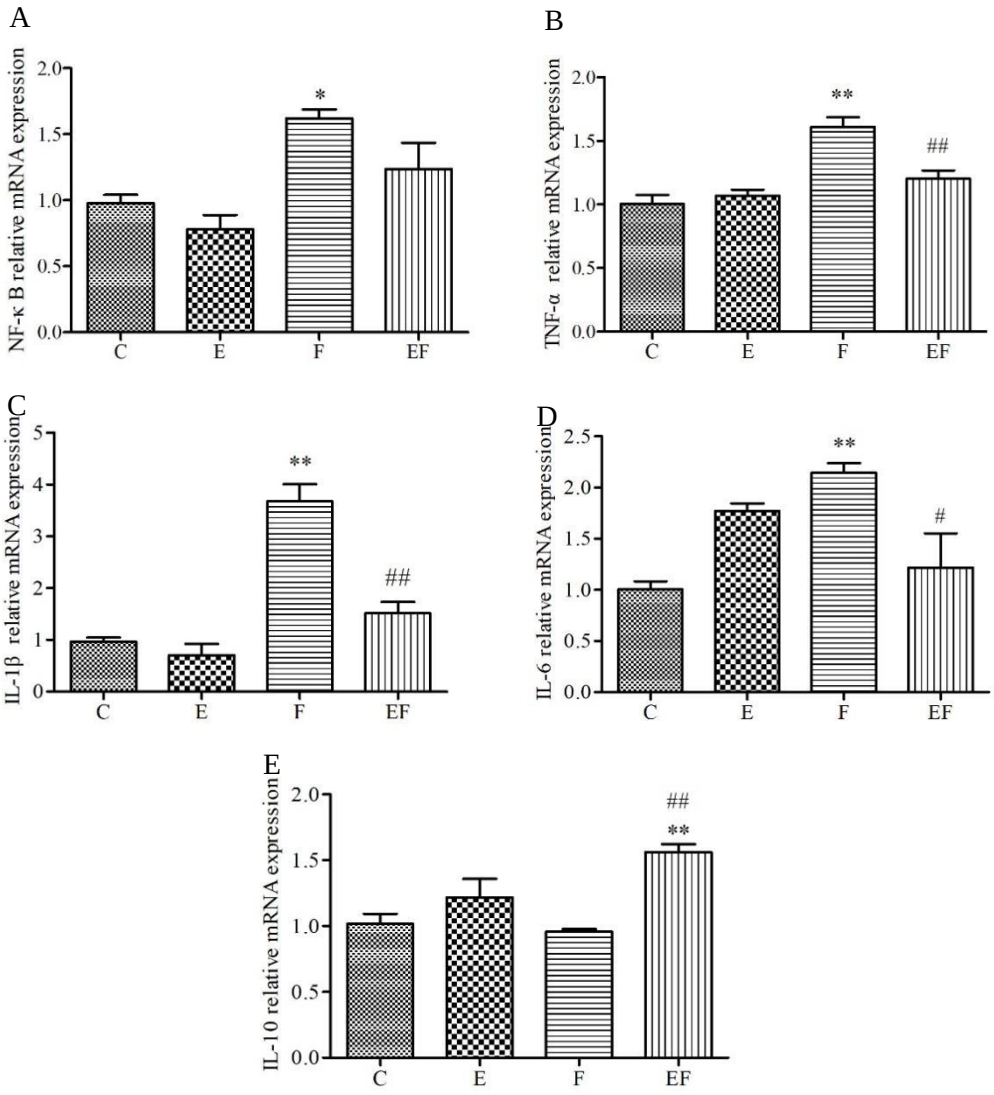

Figure 5. The mRNA levels of NF-κB, TNF-α, IL-6, IL-10, and IL-1β.

"*" or "**" indicates a statistically significant difference compared to group C (P<0.05; P<0.01).
"#" or "##" indicates a statistically significant difference compared to group F (P<0.05; P<0.01).

DISCUSSION

The incidence of cardiovascular dysfunctions is associated with excessive fluoride ingestion, manifesting as arrhythmias, decreased cardiac output, and heart block. Previous findings have indicated that fluoride exposure impairs cardiac function by inhibiting cell proliferation, inducing apoptosis, generating excessive ROS, and inducing myocardial oxidative stress and inflammatory responses.[10,24] However, to date, there is no successful treatment strategy for fluoride-induced cardiac dysfunction. Our recent studies have demonstrated the positive effects of exercise on brain function and intestinal homeostasis in fluoride-treated mice.[19,20] Therefore, the mitigating effects of exercise on heart tissue injury and the expression of inflammatory factors in mice exposed to fluoride were focused on in this study.

High concentrations of fluoride exposure can generate pathological effects in heart tissue, leading to cardiac cell damage.[25] It has been suggested that fluoride exposure resulted in irregularly arranged cardiomyocytes and altered nuclear morphology.[9] The ultrastructural analysis of cardiac tissue revealed myocardial fiber breakage and mitochondrial dissolution, characterized by fuzzy and loose mitochondrial cristae.[8,9] In the present study, results from HE staining and TEM demonstrated irregular and incomplete myofilaments, cracked Z-lines, slightly dissolved mitochondria, and fuzzy mitochondrial ridges in the fluoride group, which aligned with previous research findings. These results from histopathological

examination provided compelling evidence for the toxicity of fluoride on heart tissue. He et al. found that exercised mice exhibited ordered myocardial fibers with comparatively longer transverse diameters, decreased inflammatory cell infiltration, and less cardiomyocyte nuclear condensation.[26] Interestingly, in our study, the morphology of cardiac tissue in mice treated with both exercise and fluoride showed marked improvement, suggesting that exercise alleviated the morphological damage in cardiac tissue caused by fluoride exposure.

The heart-related enzymes AST and CK are highly sensitive indicators of cardiac damage and are widely utilized in clinical diagnostics. A previous study found that high fluoride exposure significantly enhanced the activities of AST and CK in rat heart tissues.[9] In this study, the markedly increased activities of CK and AST in fluoride-treated mice were consistent with these findings, confirming the detrimental effects of fluoride exposure on mouse heart tissue.

Moreover, CD68, a key marker of macrophages, has been interpreted as evidence of proliferating macrophages.[27,28] Macrophages serve as the first line of defense against external invaders and are critical cellular mediators of innate immune defense, producing antimicrobial substances and various cytokines.[29] It plays a vital role in maintaining cardiac homeostasis under normal conditions and in tissue repair following injury.[30] The tissue repair functions of macrophages primarily involve phagocytosis to eliminate unwanted substances.[31] The effects of myocardial macrophages on supporting cardiac homeostasis are closely associated with the activation of the inflammasome and the release of inflammatory factors in myocardial tissue.[32] In this study, the expression of CD68 in the fluoride group was higher than that in the control group, indicating an inflammatory response of fluoride on myocardial tissue. This result aligned with earlier studies showing that high fluoride exposure leads to upregulated CD68 levels, which promoted phagocytosis in cells.[27] Ample evidence indicated that prolonged exercise markedly reduces the number of bacterially induced macrophages.[33] Similarly, our results from immunofluorescence staining demonstrated that fluoride-increased CD68 expression was downregulated in the exercise-plus-fluoride group, preliminarily suggesting that exercise alleviated the cardiac inflammatory response elicited by fluoride.

It is widely acknowledged that exposure to fluoride enhances inflammatory reactions.[34] NF-κB, a transcription factor, regulates the expression of pro-inflammatory cytokines and is critical for immune and inflammatory responses.[35] The activation of NF-κB is associated with the production of pro-inflammatory mediators, including IL-1β, IL-6, IL-10, and TNF-α.[36] IL-1β serves as a typical early-response pro-inflammatory cytokine, playing crucial roles in the maintenance and development of inflammation.[37] Additionally, IL-6 and TNF-α contribute to systemic inflammation as pro-inflammatory cytokines.[13] Elevated expressions of IL-6 and TNF-α have been reported to increase the risk of various cardiac diseases, including heart failure, cardiac hypertrophy, and fibrosis, and are recognized as major factors in chronic inflammatory responses.[38,39] Conversely, IL-10 functions as an anti-inflammatory cytokine that plays a protective role in cardiac tissues.[40] Related studies have demonstrated that NaF treatment significantly upregulates the levels of NF-κB, IL-1β, IL-6, and TNF-α, while downregulating the expression of IL-10.[41] In the current study, the mRNA levels of NF-κB, IL-1β, IL-6, and TNF-α were significantly elevated in the F group, suggesting that fluoride exposure induced an inflammatory response in the hearts of mice. However, there were no significant changes observed in the anti-inflammatory cytokine IL-10, which may be attributed to an unbalanced dynamic between anti-inflammatory and pro-inflammatory factors. Aerobic exercise training has been recommended as a potential anti-inflammatory therapy.[42] It has been reported that physical activity can decrease the levels of cytokines such as NF-κB, IL-6, IL-1β, and TNF-α,[19,43] in addition to elevate IL-10 levels. The exercise training in the present experiment also reduced the levels of IL-6, IL-1β, and TNF-α and enhanced the expression of IL-10, suggesting that exercise training may reduce inflammation.

CONCLUSIONS

In conclusion, the current findings indicated that exercise mitigated cardiac morphological lesions, increased the number of macrophages, inhibited the mRNA expressions of pro-inflammatory factors IL-6, IL-1β, and TNF-α, and elevated the level of the anti-inflammatory factor IL-10 in fluoride-exposed mice, which provided new evidence regarding the preventive effects of exercise on fluoride-induced cardiotoxicity.

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CONFLICT OF INTERESTS

The authors declare no competing interests.

REFERENCES

[1] Buzalaf MA. Review of fluoride intake and appropriateness of current guidelines. Adv Dent Res 2018;29:157-166.

[2] Sun Z, Niu R, Wang B, Jiao Z, Wang J, Zhang J, et al. Fluoride-induced apoptosis and gene expression profiling in mice sperm in vivo. Arch Toxicol 2011;85:1441-1452.

[3] Niu R, Chen H, Manthari RK, Sun Z, Wang J, Zhang J, et al. Effects of fluoride on synapse morphology and myelin damage in mouse hippocampus. Chemosphere 2018;194:628-633.

[4] Liu J, Wang HW, Lin L, Miao CY, Zhang Y, Zhou BH. Intestinal barrier damage involved in intestinal microflora changes in fluoride-induced mice. Chemosphere 2019;234:409-418.

[5] Ma Y, Niu R, Sun Z, Wang J, Luo G, Zhang J, et al. Inflammatory responses induced by fluoride and arsenic at toxic concentration in rabbit aorta. Arch Toxicol 2012;86:849-856.

[6] Flora SJ, Pachauri V, Mittal M, Kumar D. Interactive effect of arsenic and fluoride on cardio-respiratory disorders in male rats: possible role of reactive oxygen species. Biometals 2011;24:615-628.

[7] Quadri JA, Sarwar S, Pinky, Kar P, Singh S, Mallick SR, et al. Fluoride induced tissue hypercalcemia, IL-17 mediated inflammation and apoptosis lead to cardiomyopathy: Ultrastructural and biochemical findings. Toxicology 2018;406-407:44-57.

[8] Wang HW, Liu J, Zhao J, Lin L, Zhao LW, Tan PP, et al. Ca(2+) metabolic disorder and abnormal expression of cardiac troponin involved in fluoride-induced cardiomyocyte damage. Chemosphere 2018;201:564-570.

[9] Yan X, Dong N, Hao X, Xing Y, Tian X, Feng J, et al. Comparative transcriptomics reveals the role of the Toll-like receptor signaling pathway in fluoride-induced cardiotoxicity. J Agric Food Chem 2019;67:5033-5042.

[10] Xie J, Yan X, Xu G, Tian X, Dong N, Feng J, et al. ITRAQ-based proteomics reveals the potential mechanism of fluoride-induced myocardial contraction function damage. Ecotoxicol Environ Saf 2020;197:110605.

[11] Nishida K, OtsuK. Inflammation and metabolic cardiomyopathy. Cardiovasc Res 2017;113:389-398.

[12] Wang HW, Zhao WP, Liu J, Tan PP, Tian WS, Zhou BH. ATP5J and ATP5H proactive expression correlates with cardiomyocyte mitochondrial dysfunction induced by fluoride. Biol Trace Elem Res 2017;180:63-69.

[13] Panneerselvam L, Raghunath A, Sundarraj K, Perumal E. Acute fluoride exposure alters myocardial redox and inflammatory markers in rats. Mol Biol Rep 2019;46:6155-6164.

[14] Chen X, Li H, Wang K, Liang X, Wang W, Hu X, et al. Aerobic exercise ameliorates myocardial inflammation, fibrosis and apoptosis in high-fat-diet rats by inhibiting P2X7 purinergic receptors. Front Physiol 2019;10:1286.

[15] Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985) 2005;98:1154-1162.

[16] Lira FS, Koyama CH, Yamashita AS, Rosa JC, Zanchi NE, Batista ML, et al. Chronic exercise decreases cytokine production in healthy rat skeletal muscle. Cell Biochem Funct 2009;27:458-461.

[17] Fuchs M, Drexler H. Chronic heart failure and proinflammatory cytokines: possible role of physical exercise. Exerc Immunol Rev 2004;10:56-65.

[18] Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest 2017;47:600-611.

[19] Fu R, Niu R, Zhao F, Wang J, Cao Q, Yu Y, et al. Exercise alleviated intestinal damage and microbial disturbances in mice exposed to fluoride. Chemosphere 2022;288:132658.

[20] Cao Q, Wang J, Hao Y, Zhao F, Fu R, Yu Y, et al. Exercise ameliorates fluoride-induced anxiety- and depression-like behavior in mice: role of GABA. Biol Trace Elem Res 2022;200:678-688.

[21] Swain DP, Franklin BA. VO(2) reserve and the minimal intensity for improving cardiorespiratory fitness. Med Sci Sports Exerc 2002;34(1):152-157.

[22] Zhang J, Guo Y, Wang Y, Song L, Zhang R, Du Y. Long-term treadmill exercise attenuates Aβ burdens and astrocyte activation in APP/PS1 mouse model of Alzheimer's disease. Neurosci Lett 2018;666:70-77.

[23] Myers J. Cardiology patient pages. Exercise and cardiovascular health. Circulation. 2003;107(1):e2-5.

[24] Panneerselvam L, Govindarajan V, Ameeramja J, Nair HR, Perumal E. Single oral acute fluoride exposure causes changes in cardiac expression of oxidant and antioxidant enzymes, apoptotic and necrotic markers in male rats. Biochimie 2015;119:27-35.

[25] Yan X, Wang L, Yang X, Qiu Y, Tian X, Lv Y, et al. Fluoride induces apoptosis in H9c2 cardiomyocytes via the mitochondrial pathway. Chemosphere 2017;182:159-165.

[26] He W, Tang Y, Li C, Zhang X, Huang S, Tan B, et al. Exercise enhanced cardiac function in mice with radiation-induced heart disease via the FNDC5/Irisin-dependent mitochondrial turnover pathway. Front Physiol 2021;12:739485.

[27] Yang T, Zhang Y, Zheng D, Hao Y, Snead ML, Duan X. High-fluoride promoted phagocytosis-induced apoptosis in a matured ameloblast-like cell line. Arch Oral Biol 2015;60:84-90.

[28] Swirski FK, Robbins CS, Nahrendorf M. Development and function of arterial and cardiac macrophages. Trends Immunol 2016;37:32-40.

[29] Kizaki T, Takemasa T, Sakurai T, Izawa T, Hanawa T, Kamiya S, et al. Adaptation of macrophages to exercise training improves innate immunity. Biochem Biophys Res Commun 2008;372:152-156.

[30] Ma Y, Mouton AJ, Lindsey ML. Cardiac macrophage biology in the steady-state heart, the aging heart, and following myocardial infarction. Transl Res 2018;191:15-28.

[31] Gordon S. Phagocytosis: an immunobiologic process. Immunity 2016;44:463-475.

[32] Nicolás-Ávila JA, Lechuga-Vieco AV, Esteban-Martínez L, Sánchez-Díaz M, Díaz-García E, Santiago DJ, et al. A network of macrophages supports mitochondrial homeostasis in the heart. Cell 2020;183:94-109.e123.

[33] Woods J, Lu Q, Ceddia MA, Lowder T. Special feature for the Olympics: effects of exercise on the immune system: exercise-induced modulation of macrophage function. Immunol Cell Biol 2000;78:545-553.

[34] Gutowska I, Baranowska-Bosiacka I, Goschorska M, Kolasa A, Łukomska A, Jakubczyk K, et al. Fluoride as a factor initiating and potentiating inflammation in THP1 differentiated monocytes/macrophages. Toxicol In Vitro 2015;29:1661-1668.

[35] Jayandharan GR, Aslanidi G, Martino AT, Jahn SC, Perrin GQ, Herzog RW, et al. Activation of the NF-kappaB pathway by adeno-associated virus (AAV) vectors and its implications in immune response and gene therapy. Proc Natl Acad Sci U S A 2011;108:3743-3748.

[36] Wang Z, Jiang W, Zhang Z, Qian M, Du B. Nitidine chloride inhibits LPS-induced inflammatory cytokines production via MAPK and NF-kappaB pathway in RAW 264.7 cells. J Ethnopharmacol 2012;144:145-150.

[37] Shin DS, Kim KW, Chung HY, Yoon S, Moon JO. Effect of sinapic acid against carbon tetrachloride-induced acute hepatic injury in rats. Arch Pharm Res 2013;36:626-633.

[38] Kanda T, Takahashi T. Interleukin-6 and cardiovascular diseases. Jpn Heart J 2004;45:183-193.

[39] Adali MK, Varol E, Aksoy F, Icli A, Ersoy IH, Ozaydin M, et al. Impaired heart rate recovery in patients with endemic fluorosis. Biol Trace Elem Res 2013;152:310-315.

[40] Kwon WY, Cha HN, Heo JY, Choi JH, Jang BI, Lee IK, et al. Interleukin-10 deficiency aggravates angiotensin II-induced cardiac remodeling in mice. Life Sci 2016;146:214-221.

[41] Luo Q, Cui H, Deng H, Kuang P, Liu H, Lu Y, et al. Sodium fluoride induces renal inflammatory responses by activating NF-κB signaling pathway and reducing anti-inflammatory cytokine expression in mice. Oncotarget 2017;8:80192-80207.

[42] Ploeger HE, Takken T, de Greef MH, Timmons BW. The effects of acute and chronic exercise on inflammatory markers in children and adults with a chronic inflammatory disease: a systematic review. Exerc Immunol Rev 2009;15:6-41.

[43] Wang J, Lu C, Zheng L, Zhang J. Peripheral Inflammatory Biomarkers of methamphetamine withdrawal patients based on the neuro-inflammation hypothesis: the possible improvement effect of exercise. Front Psychiatry 2021;12:795073.