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ORIGINAL ARTICLE

# The hepatoprotective effect of sodium butyrate on hepatic inflammatory injury mediated by the NLRP3 inflammatory pathway in subchronic fluoride-exposed mice

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## Abstract

**Background** Excessive subchronic fluoride exposure can cause severe damage to detoxification organs, including the liver. Sodium butyrate has anti-inflammatory, antitumor, antioxidant and immunomodulatory properties. However, relatively few studies have investigated the effects of sodium butyrate on liver injury caused by subchronic fluoride exposure. The purpose of this research was to investigate the effect and mechanism of sodium butyrate on fluoride-induced hepatic inflammatory injury via the expression of nod-like receptor protein 3 (NLRP3).

**Methods** Mice were subjected to randomization into four groups, control group (C), fluorosis group (F), sodium butyrate alone group (S), and treatment group (Y). The mice in groups F and F + S drank 100 mg/L sodium fluoride-containing distilled water freely every day. After fluoride exposure lasted for 3 months, the mice in group S and F + S were gavaged with sodium butyrate daily at a concentration of 1000 mg/kg. Following the treatment regimen, liver specimens were collected for analysis. The mRNA and protein expression levels of inflammatory factors and NLRP3 and its downstream gene were measured by RT-qPCR and western blotting.

**Results** The histological hematoxylin and eosin (H&E) staining of liver showed that the subchronic fluoride-exposed group were chronic inflammation. The liver of treatment group were less vacuolar degeneration and inflammatory infiltration. The results of the biochemical assay showed that the subchronic fluoride-exposed group were liver injury. In addition, the detection of oxidative stress indicators showed that chronic subchronic fluoride exposure could lead to an increase in the level of oxidative stress in the liver, and the treatment alleviated this increase. RT-qPCR results showed that compared with those in the control group, the mRNA levels of the inflammatory factors TNF-α, IL-6 and IL-1β, the NLRP3 inflammasome and its downstream factors NLRP3, caspase-1, gasdermin D (GSDMD) and IL-18 increased in the liver tissue of mice in the subchronic fluoride-exposed group. Sodium butyrate released inflammatory factors during subchronic fluoride exposure and inhibited the protein expression of activated NLRP3 to a certain extent.

**Conclusions** Sodium butyrate may play a protective role by antagonizing the production of activated inflammasomes and their downstream inflammatory factors in the livers of subchronic fluoride-exposed mice.

**Keywords** Sodium butyrate · Fluorine · Hepatic injury · NLRP3 inflammation pathway

## Introduction

Fluoride is a relatively active chemical element in nature. Excessive fluoride can cause severe damage to several vital organs, including the liver [1], testicle [2], kidney [3], and brain [4]. Its toxic effects mainly involve DNA oxidative damage, oxidative stress and cell apoptosis [5–7]. Previously studies indicate that excessive subchronic fluoride exposure may cause liver damage by interfering with detoxification ability and metabolic processes [8]. Drinking water

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Extended author information available on the last page of the article with fluoride ion concentrations exceeding 2.0 mg/L can cause significant liver function damage [9, 10]. In animal experiments, chronic subchronic fluoride exposure can lead to mild structural disorder of liver tissue in pathological sections, unclear cell boundaries, and mild damage to the nucleus; ultrastructural changes in liver cells are observable under an electron microscope, showing damage to the nuclear membrane and organelles, such as endoplasmic reticulum expansion, mitochondrial membrane blurring, and increased vacuolar degeneration [11, 12]. In addition, chronic subchronic fluoride exposure leads to a decrease in total protein and albumin levels in animal serum and an increase in indicators such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [13].

Butyric acid is a short-chain fatty acid (SCFA) that occurs naturally in butter and cheese [14] and can also be produced by fermentation of dietary fiber by endogenous microorganisms in the intestine [15]. Previous studies have shown that butyrate has anti-inflammatory pharmacological activity in various tissues [16–19]. Notably, butyrate not only participates in various biological processes of the gut-liver axis but also appropriately regulates the inflammatory response of the liver and improves its metabolic balance and peroxidation status. Studies have shown that in the liver, butyrate inhibits the expression of Toll-like receptor 4 (TLR4) and the activation of nuclear factor kappa B (NF-κB) and attenuates the infiltration of macrophages [20, 21]. Moreover, supplementation with sodium butyrate can significantly improve nonalcoholic fatty liver disease (NAFLD) [22], and this conclusion has also been proven in animal experiments [23]. Regarding the regulatory effect of sodium butyrate on the abnormal activation of NLRP3, Jiang et al. [24] found that in bovine macrophages, sodium butyrate can reduce the inflammatory response caused by LPS by inhibiting the NF-κB and NLRP3 signaling pathways. Moreover, in animal experiments, sodium butyrate can effectively inhibit the activation of NLRP3 in the intestine, thereby reducing $H_2O_2$-induced intestinal inflammation [25]. However, whether sodium butyrate protects against fluoride-induced liver injury and what the underlying mechanisms may have not been thoroughly studied.

The purpose of this study was to investigate whether subchronic fluoride exposure can lead to the activation of NLRP3 inflammasomes and explore the effect of sodium butyrate on liver injury and NLRP3 inflammasomes in subchronic fluoride-exposed mice.

## Materials and methods

### Experimental materials

#### Experimental design

SPF ICR 4-week-old mice ranging from 20 to 25 g were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Shenyang, China). The animals were raised at the Experimental Animal Center of Shenyang Medical College and acclimated for 1 week under good laboratory conditions (22–26 °C, 50 ± 5% relative humidity, normal light, and light/dark cycle of 12 h). During the treatment, the animals were allowed free access to food and water. All experiments were conducted following the recommendations of the Shenyang Medical College Animal Ethical Committee.

Mice were randomized into four groups, with each group containing ten animals after acclimatization: the control group (C), subchronic fluoride exposure group (F), sodium butyrate group (S), and subchronic fluoride exposure + 1000 mg/kg sodium butyrate treatment group (Y). Each mouse in the C and S groups freely drank distilled water, and the F and Y groups freely drank 100 mg/L fluorinated distilled water (Sigma-Aldrich, St. Louis, MO, USA). The dosage of fluoride and sodium butyrate administered was determined in a preexperiment. After three months of exposure, the mice in the S and Y groups were given 1000 mg/kg sodium butyrate solution(Macklin, Beijing, China) daily by gavage for eight weeks, while the mice in the C and F groups were given the same dose of physiological saline. The body weight and general condition of the mice were observed regularly.

### Experimental methods

After eight weeks of treatment, all the experimental mice were euthanised by cervical dislocation according to standard methods. The mice were weighed before sacrifice. The livers of each group were rinsed with saline to remove surface blood, and the excess water was dried with filter paper, then the livers of each group were weighed on an accurate balance. The liver coefficient (%) was calculated as the ratio of liver weight to body weight.

#### Fluoride ion selective electrode method

The fluoride concentrations in the urine and blood were measured according to the People's Republic of China Health Industry Standard (WS/T 89-2015 and WS/T 212–2001) [33, 34]. The mice were placed in a metabolic cage and deprived of water for 24 h to collect urine. Subsequently, the mice were euthanised by cervical dislocation, and blood was collected from the orbit. Blood samples were centrifuged (3000 rpm, 10 min) to obtain serum. The ionometer (Leici Instrument, Shanghai, China) was calibrated with standard solutions of different concentrations of fluoride. The sample and total ion concentration buffer (Leici Instrument, Shanghai, China) were added at a ratio of 1:1, and the mixture was placed under a fluoride ion meter for 30 s to measure the mean concentration.

### Detection of ALT and AST in liver tissue and serum

Mouse plasma (5 µL) was collected via a pipette, and a precision balance was used to accurately weigh 30–50 mg of mouse liver tissue in a 2 ml Eppendorf (EP) tube according to the manufacturer's instructions. The ratio of tissue weight (g) to physiological saline (ml) used was 1:9. Precooled physiological saline was added. To ensure sufficient homogenization, scissors were used to cut the tissue into small pieces. Afterward, the tissue was homogenized using a high-throughput tissue crusher, incubated for 5 min, and centrifuged at 8000 rpm for 10 min, then the supernatant was collected for analysis. ALT and AST detection kits(Nanjing Jiancheng Bioengineering Institute, China) were used to measure the levels of ALT and AST in mouse plasma and liver tissue.

### Determination of H2O2 and GST in liver tissue

The liver tissues of all the groups were homogenized, and $H_2O_2$ and GST detection kits(Nanjing Jiancheng Bioengineering Institute, China) were used to measure the levels of $H_2O_2$ and GST in the tissue.The content of $H_2O_2$ and GSH was measured at 405 nm using the standard concentration solution in the kit and the enzyme marker.

### Histological hematoxylin and eosin (H&E) staining

The liver tissue was trimmed and rinsed with water for 4 h. Alcohol gradient dehydration was performed, and the dissolved paraffin was infiltrated into the tissue blocks, then became hard wax blocks after cooling. The wax blocks were placed on a slicer (Leica, Heidelberg, Germany), cut into 4 mm slices, and transferred to slides. The wax blocks were placed in a temperature box at 60 °C for 2 h and then dried. After the paraffin sections were dehydrated, hematoxylin (Solarbio, Beijing, China) was added, and the sections were soaked for 5 min. Then, the sections were rinsed with distilled water, soaked in eosin (Solarbio, Beijing, China) for 3 min, and sealed with neutral gum. Pathological changes of livers were assessed by microscopy (Olympus, Tokyo, Japan).

**Table 1** SYBR qPCR reaction system

| Reagent | Dosage (µL) |
|---|---|
| 2×ChamQ Unversal SYBR qPCR Master Mix | 10 |
| Primer 1 (10 µM) | 0.4 |
| Primer 2 (10 µM) | 0.4 |
| cDNA | 0.2 |
| Rnase Free dd H2O | Up to 20 µL |

**Table 2** Correlation sequence of each primer

| Gene | Forward primer | Reverse primer |
|---|---|---|
| Il-18 | GCAGTGGTTTCAGCTGGG | CACACCACAGG GGAGAAGTG |
| Il-6 | TTCCATCCAGTTGCCTT CTTG | AATTAAGCCTCC GGACTTGTGAA |
| Tnf-α | CCCCAAAGGGATGAGAA GGTTC | GGCTTGTCACTC GAATTTTGAGA |
| Gapdh | AAGAGATGGGAATGTTG GCTG | CTCCCTGCATGA CTTTGTTGTC |
| Nlrp3 | GCCTTGAAGAAGAGTGG ATGC | CTGCGTGTAGC GACTGTTG |
| Gsdmd | GATCAAGGAGGTAAGC GGCA | CACTCCGGTTCT GGTTCTGG |
| Caspase-1 | GGACCCTCAAGTTTTGC CCT | AACTTGAGCTC CAACCCTCG |
| Il-1β | GAAATGCCACCTTTTGA CAGTG | TGGATGCTCTCA TCAGGACAG |

### RNA extraction and quantitative real-time polymerase chain reaction (RT–qPCR)

Total RNA was extracted with TRIzol and reverse transcribed to obtain cDNA. RT–qPCR was performed according to the manufacturer's instructions using a StepOne real-time PCR instrument (Bio-Rad) and a SYBR Premix Ex TaqII kit (Takara Bio, Shiga, Japan).The reaction system and the correlation sequence of each primer are as listed in Tables 1 and 2.

### Western blotting

The liver tissues of all groups were homogenized and the total protein concentration was measured using a BCA protein quantitation kit (Dingguochangsheng Biotechnology, Senyang, China). The proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Dingguochangsheng Biotechnology) and transferred to PVDF membranes (Millipore, Billerica, MA, USA) by Trans-Blot Turbo (Hercules, CA, USA) at a constant voltage of 100 V for 1 h. The tris-buffered saline Tween (TBST)-diluted primary antibodies used were as follows: anti-IL-6 (1:500, Wanleibio, Shenyang, China), anti-IL-1β (1:1000, Wanleibio), anti-TNF-α (1:1000, Abclone, Wuhan, China), anti-NLRP3 (1:1000, Abclone), anti-GSDMD (1:1000, Abclone), anti-β-actin (1:5000, Abclone, Wuhan, China),

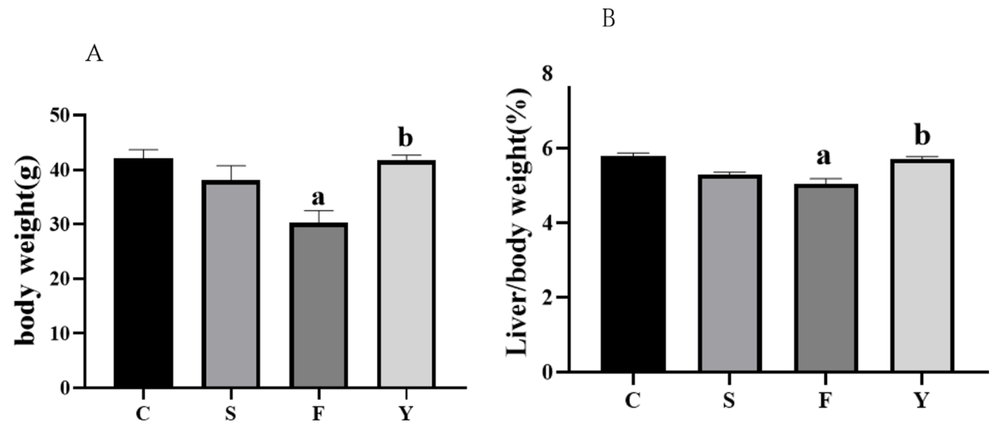


**Fig. 1** Changes in body weights (**A**) and liver coefficients (**B**) in each group. Compared with those in Group C and Group S, the body weights and liver coefficients of the mice in Group F decreased ($p<0.05$). After treatment with sodium butyrate, the body weights and liver coefficients of mice in Group Y were restored to a certain extent ($p<0.05$). The data are shown as the mean ± SEM ($n=8$). **a**, The difference was significant, compared with that in Group C ($P<0.05$); **b**, the difference was significant, compared with that in Group F ($P<0.05$)

anti-GAPDH (1:5000, Abclone) and anti-IL-18 (1:1000, Abclone). The membrane was incubated with the primary antibody at 4 °C for 12 h and then incubated with a HRP-conjugated goat anti-rabbit IgG secondary antibody (1:6000, Wuhan, Abclone). ImageJ 1.4 (Bethesda, Maryland, USA) was used to analyze the intensity of the bands.

### Statistical analysis

SPSS 22.0 statistical software was used for data analysis, and all values were expressed as the mean ± standard error of the mean (SEM). One-way ANOVA followed by Tukey's post hoc test was used for comparison of multiple groups. Statistical graphs were generated by GraphPad Prism 8 software, A *p*-value less than 0.05 was considered statistically significant.

## Results

### Mouse body weight and liver coefficients

After subchronic fluoride exposure for five months, the mice in Group F exhibited obvious disordered fur, loss of appetite, and drowsiness. The mice in Groups C and S had smooth hair, good appetite and rapid response. Moreover, as shown in Fig. 1, compared with those in Group C and Group S, the body weights and liver coefficients of the mice in Group F decreased to a lower degree ($p<0.05$). After treatment with sodium butyrate, the body weight and liver coefficient of mice in Group Y were restored to almost the same extent of the C group ($p<0.05$).

**Table 3** Concentrations of fluoride ions in the serum and urine of mice

| Group | Serum fluoride concentration (mg/L) | Urine fluoride concentration (mg/L) |
|---|---|---|
| C | $0.17 \pm 0.01$ | $1.36 \pm 0.14$ |
| S | $0.18 \pm 0.01$ | $1.24 \pm 0.15$ |
| F | $0.22 \pm 0.01^{a}$ | $8.97 \pm 0.12^{a}$ |
| Y | $0.18 \pm 0.01^{b}$ | $7.31 \pm 0.07^{b}$ |

### Concentrations of fluoride ions in serum and urine

The fluoride ion concentrations in the serum and urine of the mice were measured by the fluoride ion-selective electrode method to investigate whether subchronic fluoride exposure caused accumulation in the mice. The experimental results are shown in Table 3 the fluorine ions concentrations of the serum and urine of mice in Group S were similar to those in the control group ($p>0.05$). Subchronic fluoride exposure with or without sodium butyrate treatment significantly increased the fluoride ions concentrations of the urine in Group F and Group Y mice, compared with those in the control group ($p<0.05$), and the increase in the fluoride ion concentrations of the serum and urine in Group F were alleviated by treatment with sodium butyrate ($p<0.05$). The serum fluorine ions concentrations in Group Y were slightly greater than those of Group C, but the difference was not statistically significant ($p>0.05$).

The fluoride ions concentrations of the urine in Groups F and Y mice were greater than that in Group C ($P<0.05$). An increase in the concentrations of fluoride ions of the serum and urine in the fluoride exposure group was alleviated by treatment with sodium butyrate ($P<0.05$). The fluoride ions concentrations of Group Y were slightly greater than those of Group C, but the difference was not significant ($P>0.05$).

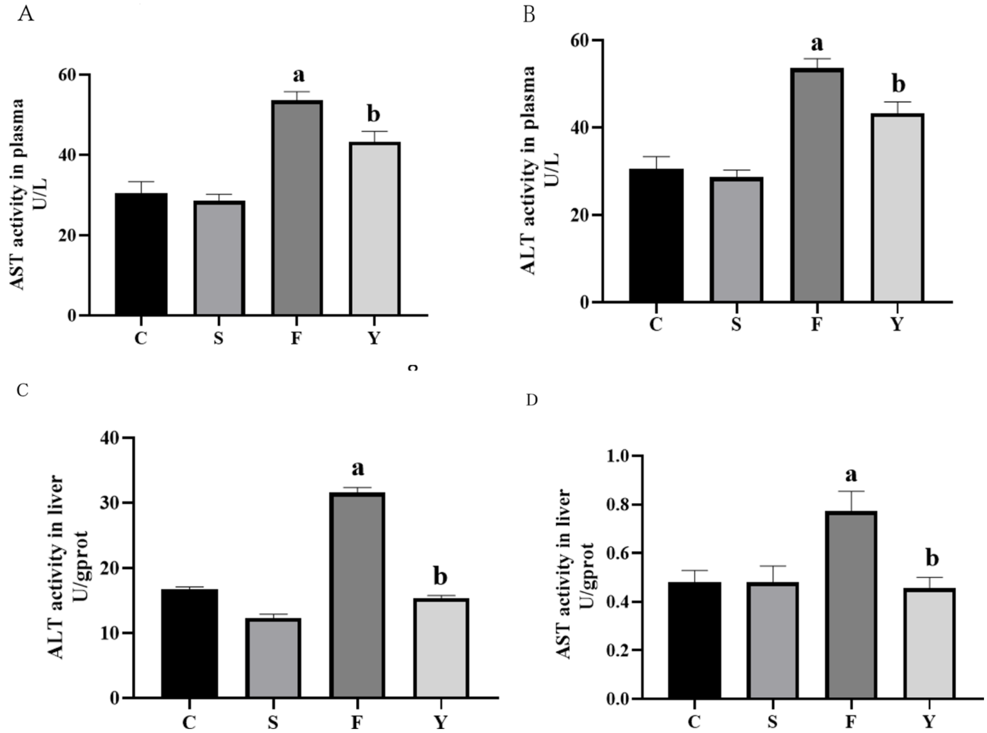


**Fig. 2** The levels of ALT (**A**, **D**) and AST (**B**, **C**) in the sera and livers in each group. Compared with those in Group C, the ALT and AST activities in the sera and livers of mice in the subchronic fluoride-exposed group tended to increase significantly ($P<0.05$). After sodium butyrate treatment, the activity of ALT and AST in Group Y mice significantly decreased ($P<0.05$). **A**, **B**: Serum transaminase, **C**, **D**: Liver transaminase. The data are shown as the mean ± SEM ($n=6$). **a**, The difference was significant, compared with that in Group C ($P<0.05$); **b**, the difference was significant. compared with that in Group F ($P<0.05$)

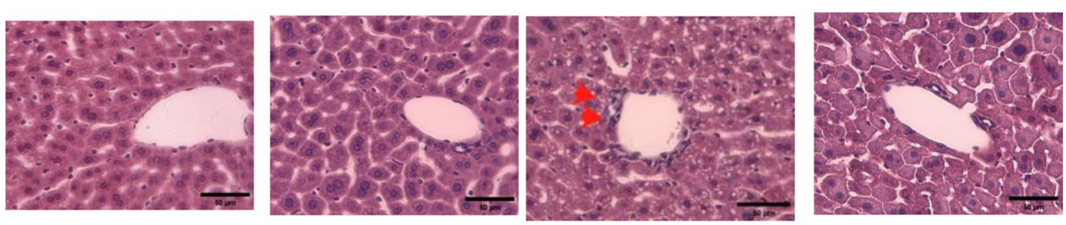


**Fig. 3** Pathological sections of liver tissues from each group stained with H&E. Scale bar: 50 μm; magnification: ×400

The data are shown as the mean ± SEM ($n=8$). a, The difference was significant, compared with that in Group C ($P<0.05$); b, the difference was significant, compared with that in Group F ($P<0.05$).

## Sodium butyrate reduces liver damage of subchronic fluoride-exposed mice

### Serum and liver transaminase levels in mice

The effects of sodium butyrate on the livers of subchronic fluoride-exposed mice were preliminarily investigated by measuring the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum and liver tissue homogenates of all groups. As shown in Fig. 2, compared with those in Group C, the serum and liver ALT and AST levels in the mice of the subchronic fluoride-exposed group tended to increase significantly ($p<0.05$). After sodium butyrate treatment, the ALT and AST levels in Group Y significantly decreased ($p<0.05$). There was no significant difference in ALT or AST levels between the S group mice and the C group.

### Mice livers pathology H&E sections

Pathological damage to the liver in the subchronic fluoride-exposed model mice was observed by histological hematoxylin and eosin staining, as shown in Fig. 3H&E staining revealed that the liver tissue structure of Group C and S mice was normal, with liver cells arranged tightly and neatly in a radial shape and no vacuoles in the cells. In Group F, the arrangement of liver cells was relatively disordered, with inflammatory cell infiltration and a large amount of vacuolar steatosis, as shown by the arrow. The inflammatory infiltration symptoms in the sodium butyrate treatment group were alleviated compared to those in the subchronic fluoride-exposed group, and the liver cells were arranged neatly with a small amount of adipose vacuoles.

### Oxidative stress levels in mice liver tissue

An oxidative stress kit was used to determine various oxidative stress factors in the liver homogenates of mice to assess oxidative stress injury in the livers of mice exposed to sodium fluoride. As shown in Fig. 4 subchronic fluoride exposure increased the level of oxidative stress in the liver. Compared with those in the C group, the $H_2O_2$ content in the liver homogenate of Group F increased, and the GSH content decreased ($p < 0.05$). The treatment with sodium butyrate in Group Y alleviated these effects ($p < 0.05$).

## Sodium butyrate antagonizes inflammatory damage of fluoride-induced liver

Cytokines are inflammatory factor involved in the inflammatory response and are expressed to certain degree in various inflammatory injuries. WB was used for assessment of IL-6, TNF-α, and IL-1β. The mRNA and protein expression levels were measured, and the results are shown in Fig. 5A-H. The analysis revealed that in Group F mice exposed to subchronic fluoride for 5 months, the mRNA and protein levels of IL-6, TNF-α, and IL-1β in the liver tissues were significantly greater compared to those in the Group C mice ($p < 0.05$), indicating that subchronic fluoride exposure can cause liver inflammatory damage to a certain extent, resulting in a large release of inflammatory factors in the tissues. The expression levels of these inflammatory factors in the liver tissue of Group Y mice were reduced ($p < 0.05$), indicating that sodium butyrate alleviated fluoride-induced inflammatory damage to a certain extent.

## Sodium butyrate antagonizes fluoride-induced NLRP3 and its downstream factors

Since long-term exposure to a large amount of fluoride can lead to the production and release of inflammatory factors in the liver, we wanted to understand the underlying mechanism involved and whether the mechanism mediates the occurrence of inflammation and can be effectively antagonized by sodium butyrate. Therefore, we studied the effect of sodium butyrate on fluoride-induced NLRP3 activation. As shown in Fig. 6A-J, both the WB and RT–qPCR results indicate that subchronic fluoride exposure can lead to an increase in the expression levels of the NLRP3 mRNA and protein compared to those in the control group ($p < 0.05$), indicating that subchronic fluoride exposure may lead to the activation of NLRP3 inflammasomes. Moreover, sodium butyrate reduced the expression level of NLRP3 ($p < 0.05$) to achieve an inhibitory effect. Therefore, we investigated the expression levels of NLRP3 downstream genes. Similar to the results for NLRP3, the mRNA expression levels of caspase-1, IL-18, and GSDMD in Group C increased ($p < 0.05$), and the Y group mice demonstrated almost the same expression levels as the control group ($p < 0.05$), which demonstrated the alleviation of the occurrence and development of NLRP3 downstream pyroptosis caused by excessive subchronic fluoride exposure. WB results showed that the protein expression levels of these factors were similar to those of the mRNA levels.

## Discussion

Endemic subchronic fluoride exposure is a chronic toxic disease caused by the accumulation of fluoride in the body due to long-term consumption or intake of fluoride exceeding physiological doses; this disease is also known as endemic

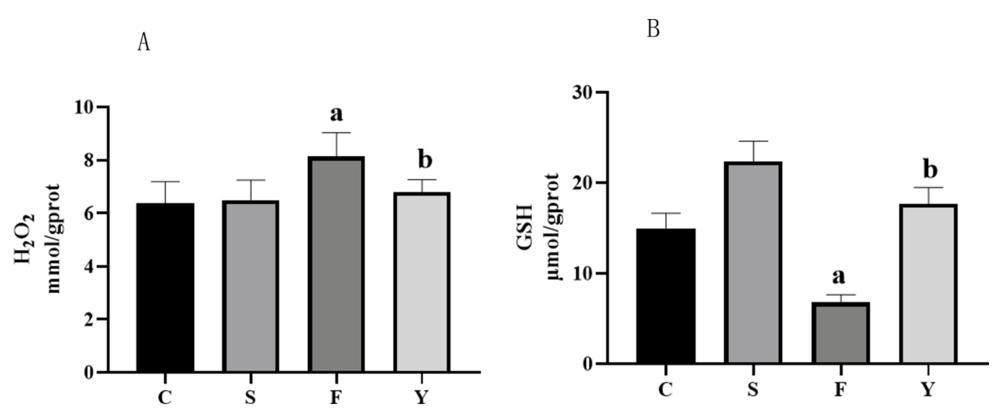


**Fig. 4** The levels of $H_2O_2$ (**A**) and GSH (**B**) in liver homogenates of each group. Compared with those in the C group, the $H_2O_2$ content in the liver homogenates of the F group increased, and the GSH content decreased ($p < 0.05$). The treatment with sodium butyrate in Group Y alleviated this effect ($p < 0.05$). The data are shown as the mean ± SEM ($n = 6$). **a**, The difference was significant, compared with that in Group C ($P < 0.05$); **b**, the difference was significant, compared with that in Group F ($P < 0.05$)

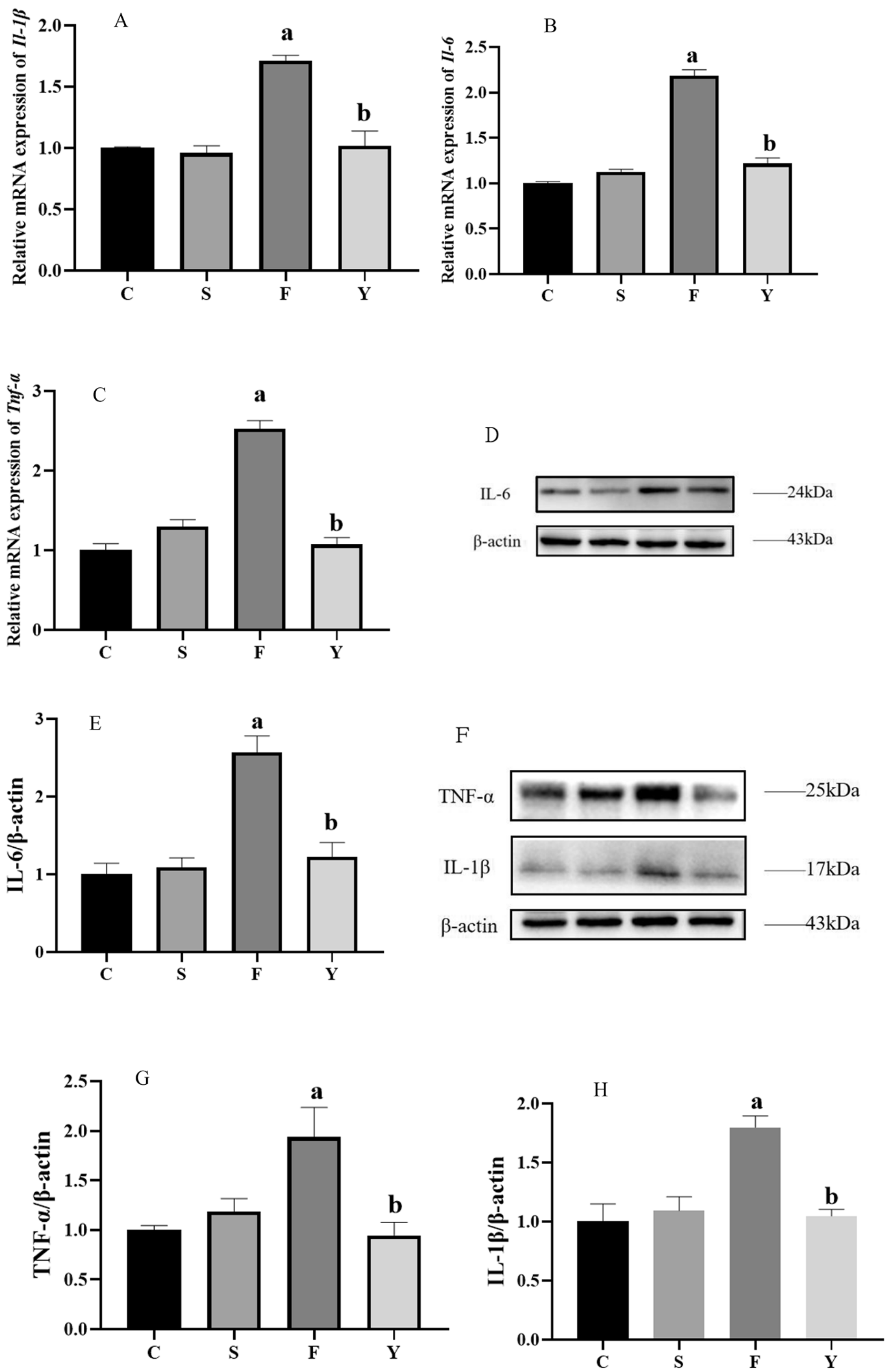


**Fig. 5** WB and RT–qPCR were used to determine the mRNA and protein levels of inflammatory factors in each group. Compared with those of Group C, the mRNA and protein levels of IL-6, TNF-α, and IL-1β in the liver tissues of mice in Group F were significantly increased ($p<0.05$), and the expression levels of these inflammatory factors in the liver tissue of Group Y mice were reduced ($p<0.05$). **A**-**C**: The mRNA expression levels of inflammatory factors. The data are shown as the mean ± SEM ($n=4$). **D**, **F**: Band diagram of the inflammatory factor proteins. **E**, **G**-**H**: Densitometric quantitative analysis of protein band patterns. The data are shown as the mean ± SEM ($n=3$). **a**, The difference was significant, compared with that in Group C ($P<0.05$); **b**, the difference was significant, compared with that in Group F ($P<0.05$)

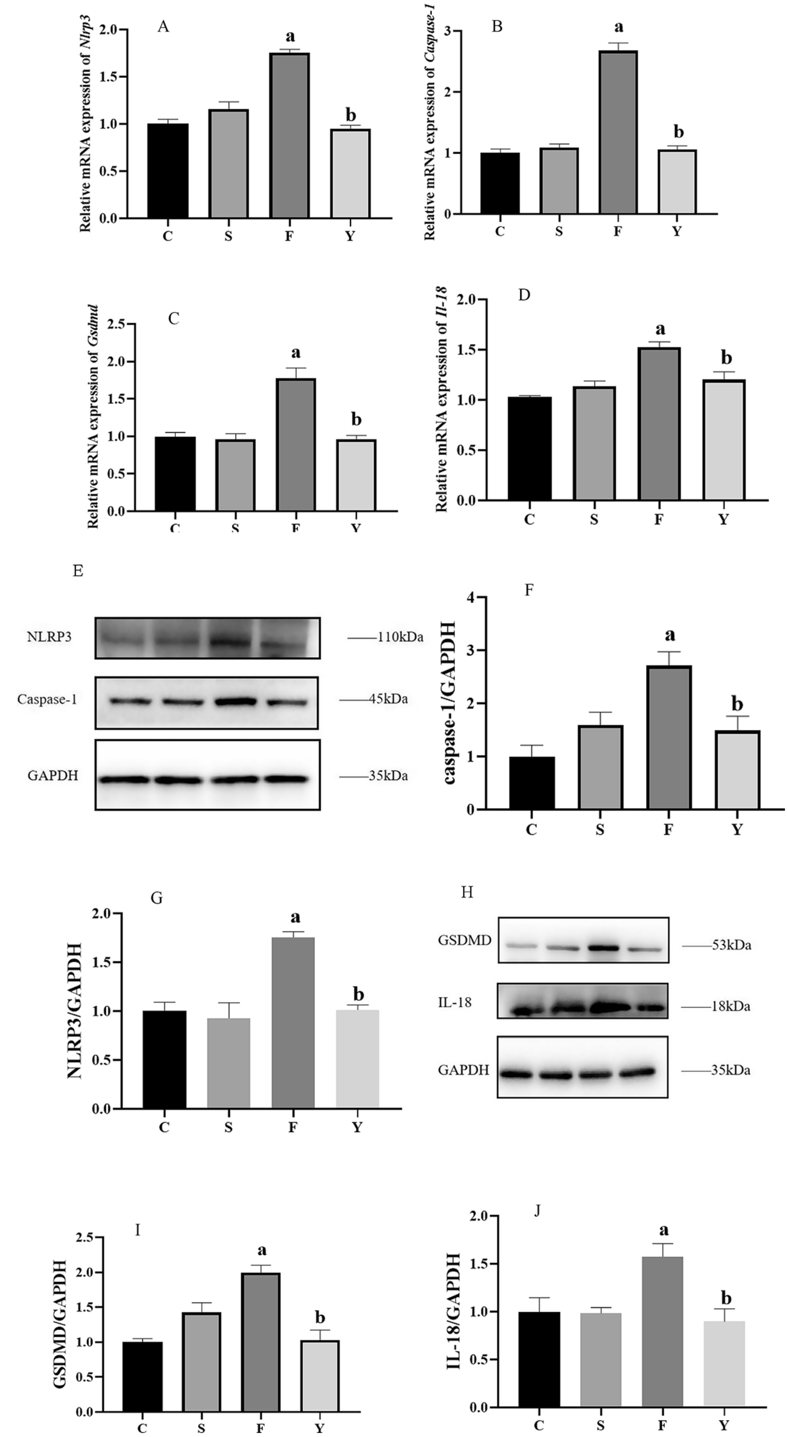


**Fig. 6** WB and RT–qPCR were used to determine the mRNA and protein levels of NLRP3 and downstream factors in each group. Compared with those in Group C, fluoride exposure led to an increase in the levels of the NLRP3 mRNA and protein ($P<0.05$). Moreover, sodium butyrate reduced the expression level of NLRP3 ($P<0.05$), thereby exerting an inhibitory effect. Similar to the NLRP3 results, compared with those in Group C, the mRNA expression levels of caspase-1, IL-18, and GSDMD were increased ($P<0.05$), while the mRNA expression levels of these genes in mice of Group Y were almost the same as those in group C. **A**-**D**: The mRNA expression levels of NLRP3 and its downstream factors. The data are shown as the mean ± SEM ($n=4$). **E**, **H**: Band chart analysis of WB detection of the protein expression levels of NLRP3 and its downstream genes; **F**-**G**, **I**-**J**: Optical density quantification of NLRP3, caspase-1, GSDMD and IL-18. The data are shown as the mean ± SEM ($n=3$). **a**, The difference was significant, compared with that in Group C ($P<0.05$); **b**, the difference was significant, compared with that in Group F ($P<0.05$)

subchronic fluoride exposure [26, 27]. Although the target organs of subchronic fluoride exposure have mainly been shown to involve teeth [28] and bones [29], recent studies have shown that subchronic fluoride exposure can still affect various nonskeletal organs and cause corresponding symptoms. This indicates that subchronic fluoride exposure can lead to systemic toxicity. Although China's targeted prevention and control policies are gradually being implemented, this type of disease has been effectively controlled in areas with severe subchronic fluoride exposure, and the number of new patients has significantly decreased. However, due to the vast territory and large population in China, the current number of affected individuals is very large. Discussing the damage caused by subchronic fluoride exposure to the morphology of nonskeletal organs and the specific pathogenesis can provide a good clinical reference for improving the public health status of endemic subchronic fluoride-exposed individuals in China. The accumulation of subchronic fluoride in the body often leads to an increase in the concentration of fluoride ions in the blood, which reaches various tissues and organs through systemic circulation, resulting in a significant increase in the content of fluorine ions in the urine of the body [30]. The liver is the largest and most important detoxifying organ in the human body and is also one of the important target organs for fluoride. Studies have shown that fluoride is metabolized through the liver, and subchronic fluoride exposure in drinking water undoubtedly imposes a heavy burden on the liver [31]. Therefore, liver damage caused by subchronic fluoride exposure has also become a hotspot in the study of nonskeletal organs. According to both epidemic and animal experimental evidence, subchronic fluoride exposure can lead to abnormal pathological changes in the morphology and function of the liver [32, 33]. Increasing evidence suggests that acetylation is a widely studied epigenetic modification that plays an important role in liver tissue diseases and liver tissue damage [34]. In recent years, histone deacetylase inhibitors have become emerging therapeutic agents for various acute and chronic liver diseases, such as NAFLD [35], liver fibrosis [36], and liver ischemia–reperfusion injury [37]. Sodium butyrate, as one of the numerous histone deacetylase inhibitors, has shown great research value in liver diseases due to its various anti-inflammatory and antitumor properties [23, 38, 39]. In this study, mice were subjected to subchronic fluoride exposure for five months, which resulted in a significant increase in fluoride ion concentrations in the serum and urine. At the same time, there were significant changes in liver pathology and transaminase levels in liver tissue homogenates and blood after subchronic fluoride exposure. The pathology results were consistent with previous conclusions, thus the liver cells of the control group mice were arranged tightly and neatly in a radial pattern, while the liver cells of the subchronic fluoride-exposed model group mice were arranged in a disorderly fashion, with inflammatory cell infiltration and fat vacuoles. The sodium butyrate treatment alleviated the pathological changes in liver tissue and reduced the infiltration of inflammatory cells. The levels of ALT/AST in the serum and liver tissue of mice subjected to subchronic fluoride exposure were also significantly increased, indicating a certain degree of damage to liver function. Moreover, there was an increase in oxidative stress levels in liver tissue after subchronic fluoride exposure, which is consistent with the conclusions of previous studies [40]. The changes in the above biochemical indicators in liver tissue of group F were alleviated by treatment with sodium butyrate. Notably, the GSH content significantly increased in the group treated solely with sodium butyrate, which may indicate the antioxidant ability of sodium butyrate.The antioxidant effect of sodium butyrate in liver tissue has been widely explored in recent years. In 2023, Zong et al. showed that the natural antifungal additive sodium butyrate could reduce oxidative stress and inflammation in liver through NR4A2-mediated histone acetylation [41]. Miao and his team found that dietary sodium butyrate supplementation had a good protective effect on the liver antioxidant function of elderly laying hens, and could improve liver injury, relieve lipid accumulation and inflammatory response [42].

In recent years, many studies have extensively explored the pathogenesis of subchronic fluoride exposure. The excessive fluoride activates the IKK β/NF-κB pathway to trigger liver inflammation, leading to liver injury [43]; exposure to fluoride leads to liver cell damage and apoptosis through mitochondrial autophagy [44]; and fluoride induces iron overload in the liver, leading to iron death [45]. However, in various recent related studies, liver inflammatory damage caused by subchronic fluoride exposure has been widely confirmed. In this study, we validated this conclusion. The present study showed that the treatment group attenuated the changes in the mRNA and protein levels of the inflammatory factors TNF-α, IL-6, and IL-1β in mice subjected to subchronic fluoride exposure, reflecting the anti-inflammatory effects of sodium butyrate on fluorine-induced liver injury. This finding is similar to that of a previous study by Deng et al., who showed that butyrate can alleviate liver inflammation by inhibiting proinflammatory macrophage activation [46]. Generally, the liver inflammatory response is an adaptive response caused by liver injury aimed at clearing harmful factors from the body. This response is accompanied by the infiltration of a large number of inflammatory cells and the release of a certain level of inflammatory factors to maintain homeostasis of the internal environment within a controllable range [47]. However, the sustained stimulation of harmful factors and injury factors can lead to the excessive release of proinflammatory cytokines and chemokines, stimulating the body to strengthen the inflammatory response and continuously cascade and expand inflammatory signals, ultimately causing damage to the body. NLRP3 inflammatory vesicles are macromolecular protein complexes in cells that are important components of intrinsic immunity and play important roles in the immune response and inflammatory propagation in organisms. The activation of NLRP3 vesicles triggers pro-caspase-1 autocleavage and promotes IL-1β production; the maturation of IL-18 and the cleavage of GSDMD lead to cellular pyroptosis [48–50], which is a new approach to the toxic mechanism of liver damage caused by subchronic fluoride exposure. Our research group determined the mRNA and protein levels of NLRP3 inflammasomes and its downstream factors caspase-1, N-GSDMD (gasdermin D), and IL-18 in subchronic fluoride-exposed mice. The levels of the relevant proteins in the mice increased to a certain extent, and sodium butyrate alleviated the inflammatory cascade caused by activation of the NLRP3 inflammasome, antagonizing the liver damage. This result confirms the antagonistic effect of sodium butyrate on other environmental pollutants, such as arsenic [51] and cadmium [52] poisoning, in previous studies.

## Conclusions

The results of this study suggest that subchronic fluoride exposure can cause pathological morphological and functional damage to the livers of mice. Sodium butyrate treatment can exert liver tissue-protective effects by inhibiting activated NLRP3 inflammasomes and their downstream proteins in the liver tissue of subchronic fluoride-exposed mice. Sodium butyrate, represents a new direction for the treatment of liver damage caused by subchronic fluoride exposure.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Compliance with ethical standards** The number of mice involved in this experiment and all the protocols were reviewed and approved by the Animal Use and Care Committee at Shenyang Medical College (protocol number: SYYXY2021031502), in accordance with the regulations and requirements of the Animal Ethics Committee and in accordance with the management regulations of experimental animals in Liaoning Province.

**Competing interests** The authors declare no competing interests.



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