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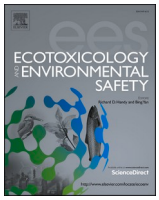

Interplay between fluorine and cadmium on intestinal accumulation, oxidative stress, permeability and inflammatory response in rats

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ABSTRACT

Fluorine (F) and Cadmium (Cd) have given rise to public concern regarding their adverse impacts on the environment and human beings. Yet, the toxic interplay between F and Cd on the intestine is still vague. Aiming to investigate the role of F on Cd-damaged intestine, a total of five groups of 30 SD rats were picked at random to be gavaged for 90 days: Control group (Ultra-pure water), Cd (Cd 1 mg/kg), Cd+LF (Cd 1 mg/kg+F 15 mg/kg), Cd+MF (Cd 1 mg/kg+F 45 mg/kg), and Cd+HF (Cd 1 mg/kg+F 75 mg/kg). It demonstrated that Cd enriched in the intestine and disordered intestinal barrier of rats. Interestingly, two side effects of F were observed resisting to the Cd toxicity. The Cd levels in colon contents were attenuated by 45.45 %, 28.11 %, and 19.54 % by F supplement, respectively. In the Cd+LF group, SOD, GSH-Px, and CAT activities elevated by 0.93, 1.76, and 1.78 times, respectively, and the MDA content reduced 0.67 times; the expressions of *NQO1*, *SOD2*, and *GSH-Px* mRNA markedly enhanced, as well as the *Keap1* mRNA significantly decreased. Nevertheless, all indexes above in the Cd+HF group showed the opposite trends. Furthermore, LPS levels decreased by 45.93 % for the Cd+LF group and increased by 12.70 % in that the Cd+HF group. The *ZO-1* expression in the Cd+LF group increased, whereas the Cd+HF group's expressions of *Claudin-1*, *Occludin*, and *ZO-1* were all diminished by 35.46 %, 27.23 %, and 16.32 %, respectively. Moreover, the levels of *TNF-α*, *IL-1β* and *TLR-4* decreased and *IL-10* level promoted, while all showed opposite trends in the Cd+HF group. Collectively, it indicated there is a twofold interplay between F and Cd on intestinal damage and mainly depends on F dosages.

1. Introduction

Cadmium (Cd) and fluorine (F) have raised public concern because of the adverse health risks they pose to the population. Cd is considered a top five carcinogen listed from the World Health Organization (Chen et al., 2019a, 2019b), and high levels of Cd in the environment can enhance the lethality rate (Zhang et al., 2020). Sewage irrigation, habitual smoking, and atmosphere formation are all of the principal sources of Cd environmental pollution (Sharma et al., 2014; Singh et al., 2017). Fluorine (F) is found in the world's soils at an average global content of approximately 321 mg/kg (Dehbandi et al., 2017). It comes mainly from natural and anthropogenic processes, including volcanic activities and agricultural emissions. Adequate F exposure is beneficial to humans as it can protect against tooth decay and improve bone and dental health, whereas excessive exposure is associated with dental and skeletal fluorosis (Adimalla and Venkatayogi, 2016). F and Cd can shape

Abbreviations: Cd, Cadmium; F, Fluorine; SD, Sprague Dawley; TISAB, Total ionic strength adjustment buffer; RT-qPCR, Real-time quantitative polymerase chain reaction; ANOVA, One-way analysis of variance; SOD, Superoxide dismutase; CAT, Catalase; GSH-Px, Glutathione peroxidase; MDA, Malondialdehyde; HQ1, Heme oxygenase-1; Keap1, Kelch-like ECH associating protein 1; NQO1, NADPH quinone oxidoreductase 1; Nrf2, Nuclear factor erythroid 2-related factor 2; LPS, Lipopolysaccharide; ZO-1, Zonula Occludens Protein 1; TNF-α, Tumor necrosis factor-alpha; IL-1β, Interleukin-1β; TLR-4, Toll-like receptor 4; IL-10, Interleukin-10.

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CdF^+ in the case of a pH value > 6.3 in the natural environment, making them easier to accumulate in animals and humans via the soil-plant system (Han et al., 2019; Singh and Bechan, 2021). Accordingly, more emphasis should be placed on the negative effects of the presence of F and Cd in the nature.

To date, co-contamination by F and Cd has been reported in many geographical regions worldwide (Li et al., 2019, 2022a, 2022b). Guizhou, a typical carbonate region, has a high level of natural geological characteristics for F and Cd, with background Cd in the soils (0.659 mg/kg) well above national background (0.097 mg/kg) and average F concentrations reaching 1200 mg/kg (Li et al., 2023a, 2023b). In the previous study, we found that rice produced non-cancer health risk to local populace when co-occurrence to F and Cd (Hazard index, HI>1.0) in Guizhou (Li et al., 2022a, 2022b). Other studies have shown that crops co-contaminated with Cd and F pose a threat to the health of the residents (Yuan et al., 2019; Yu et al., 2022). Meanwhile, the interaction between Cd and F in vivo has been a matter of concern for some scientists. Arab-Nozari et al. (2020) showed that significant increases in liver enzymes and pathological changes were recorded in rats co-treated with F and Cd when compared to their single treatment, suggesting that synergistic effects occur in combinations of F and Cd. On the contrast, one study showed that low F levels may be inversely related to increased vertebral bone mineral density following Cd treatment, suggesting an antagonistic effect between them (Chen et al., 2013). Therefore, the toxic interplay of F and Cd are still controversial and need to be further elucidated.

The intestinal tract is an important organ in regulating the body's stress imbalance and inflammatory response, as well as being an important barrier to the invasion of the body by exogenous agents. Although only 0.5–3 % of the Cd consumed might be taken up into the intestinal epithelial cells, the majority of Cd could be retained in the intestinal micro-environment to exert destructive effects (Yang et al., 2021a, 2021b). More than 60 % of F can be absorbed through the mucosa of gastrointestinal tract. Epidemiological data proved that F exposure could aggravate non-specific chronic gastrointestinal complaints, like abdominal pain, bloating, constipation, and sickness (Moparthi and Koch, 2019). Extensive studies concluded that treatment with F or Cd alone damages the intestinal permeability barrier, upsets the balance of the bacterial flora, and undermines the intestinal tight junctions (Jiang et al., 2020; Chen et al., 2013). In our previous attempt, it verified that Cd exposure perturbed the composition of gut microbiota in rats, especially decreasing the abundance of *Lactobacillus*. And low level of F could inhibit the decreasing growth of *Lactobacillus* by Cd-mediated (Li et al., 2023a, 2023b). However, the study concentrating on the intestinal flora alone is not sufficient to clarify the toxic interplay of F and Cd co-exposed in the intestinal tract.

Impaired intestinal barrier integrity may increase intestinal permeability, disrupt intestinal tight junctions, and indirectly allow other exogenous toxicants to cross the epithelial barrier. Therefore, it is significant to clarify the toxicological relationship of F and Cd in the intestinal barrier. F is well known to be a double-edged sword. Based on this factor and our previous attempt, the effects of F on Cd-induced intestinal barrier damage will be investigated by constructing different doses of F-intervened Cd-contaminated rat models, focusing on intestinal accumulation, antioxidant stress, permeability and inflammatory response in rats. As a valuable attempt, the present study may contribute to understand the dose-response relationship of F and Cd, with a view to providing scientific foundation and theoretical support for the prevention of intestinal damage co-produced by F and Cd.

2. Materials and methods

Our experimental approaches were approved for use by the Guizhou Medical University Animal Research and Use Ethics Review Board (1900947).

2.1. Experimental animal and rearing condition

A rat model was selected for sub-chronic (90-day) toxicity testing to simulate the route and duration of human exposure to F and Cd under the geological background of high levels of F and Cd. A total of 30 healthy male Sprague-Dawley (SD) rats with a weight of 220 ± 20 g were purchased at Liaoning Changsheng Biotechnology Co (License No. SCXK (Liao)) 2020–0001, China). Experimental rats were picked at random to be gavaged: Control group (Ultra-pure water), Cd (Cd 1 mg/kg), Cd+LF (Cd 1 mg/kg+F 15 mg/kg), Cd+MF (Cd 1 mg/kg+F 45 mg/kg), and Cd+HF (Cd 1 mg/kg+F 75 mg/kg), respectively. The concentrations of Cd and F have been set on the basis of the previous literature (Nair et al., 2014) and adjusted on the investigated level of the crops of Guizhou, China (Data were not shown). Cadmium chloride ($CdCl_2{\cdot}2.5H_2O$) at 99.0 % and sodium fluoride (NaF) at 99.5 % were supplied by Tianjin Kemiou Co., Ltd, China. $CdCl_2{\cdot}2.5\ H_2O$ and NaF were dissoluted and then diluted with the ultra-pure water.

At the SPF experimental laboratory, rats were maintained in a temperature-controlled environment (22 ± 5℃, 55 ± 1 %) and subjected to a 12-h cycle of light and darkness. Normal food and water were supplied autonomously.

2.2. Sample collection

On 90-day of the experiment, all rats were given free access to drinking water and fasted for 24 h. After this period, all of the rats were euthanized by an injection of phenobarbital. Subsequently, colonic tissue and contents were collected in 2 ml centrifuge bottles and rapidly frozen in nitrogen. The remaining tissue was stored at −80℃ pending further processing. All sampling steps in this experiment were performed on a ultra-clean laboratory bench.

2.3. Measurements of F and Cd

After being weighed, colon contents were acidified with 5 ml of nitric acid (95 % HNO_3) at 140°C for 4 h until the solvent was clear and discoloured. The liquid was then diluted to a volume of 10 ml using ultra-pure water. Next, Cd was quantified by Inductively Coupled Plasma Mass Spectrometry (NexION 2000 ICP-MS, PerkinElmer, USA) as previously reported (Li et al., 2023a, 2023b), and the Fluoride Selective Ion Electrode based on the potentiometric technique was applied to detect the levels of F contents (Orion, Thermo Scientific, Massachusetts, USA). Moreover, to maintain a stable ionic strengths and remove other interfering substances, sample tissues and contents were diluted with buffer for adjustment of the total ionic strength.

2.4. Serum LPS assay

Lipopolysaccharide (LPS) levels in the serum were measured by the Limulus Amebocyte Lysate Kit as instructed by the manufacturer from Xiamen biotechnology co., ltd. Samples were characterized on a microplate reader at a specific wavelength of 545 nm (BioTek, Winooski, USA) (Yu et al., 2020).

2.5. Determinations of antioxidants and inflammatory cytokines

SOD, CAT, GSH-Px and MDA were assayed using their individual kits according to methods described in (Nanjing Jiancheng Institute of Biological Engineering, China). Tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), toll-like receptor 4 (TLR-4) and interleukin-10 (IL-10) levels were measured using commercially available ELISA kits as described in the producers manual (Nanjing Jiancheng Institute of Biological Engineering, China).

2.6. RT-qPCR measurements of gene expression

The method of real-time quantitative polymerase chain reaction (RT-qPCR) was conducted to analyze the mRNA expressions of intestinal junction proteins, antioxidant genes, and inflammatory factors in colon tissues. All RNA was isolated from colon samples with the Trizol reagent as directed by the manufacturer (Wuhan service bio-Technology Co., Ltd, China). First-strand complementary DNA (cDNA) was prepared using the Prime Sequencing Reagent Kit with gDNA Eraser (Takara, Beijing, China). TB Green Premix Ex *Taq*II was then employed to assess the relative expression of genes quantified by RT-qPCR (Takara, Beijing, China). To assess the purification, the 20 μL reaction volume was determined by CFX 96 real-time PCR detection instrument (Bio-Rad, USA), which was started at 95°C for 10 min, followed by 40 cycles at 95°C for 10 s for denaturation and then annealing at 60°C for 30 s. For reference genes, β-actin was performed to standardise relative target gene expression, with values expressed as $2^{-\Delta\Delta Ct}$. Sequencing information is provided in Table S1.

2.7. Statistical analysis

The statistical tools used for analysis and graphing are SPSS 22.0 and GraphPad Prism 9.3. All data are expressed as mean ± SE (n=3). Statistical differences between experimental groups were estimated using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. The Pearson correlation analysis was performed to identify the relationships between the parameters studied. A level of $p<0.05$ is considered significantly different.

3. Results

3.1. Effects of F on Cd accumulation of colon contents

The Cd levels in colon contents were clearly increased via Cd exposure ($p<0.0001$) (Figure S1). However, the Cd concentrations were declined in all F-treated groups. Specifically, the Cd levels in colon contents were attenuated by 45.45 %, 28.11 %, and 19.54 % by F supplement and followed a downward trend, respectively (Figure S1a). In

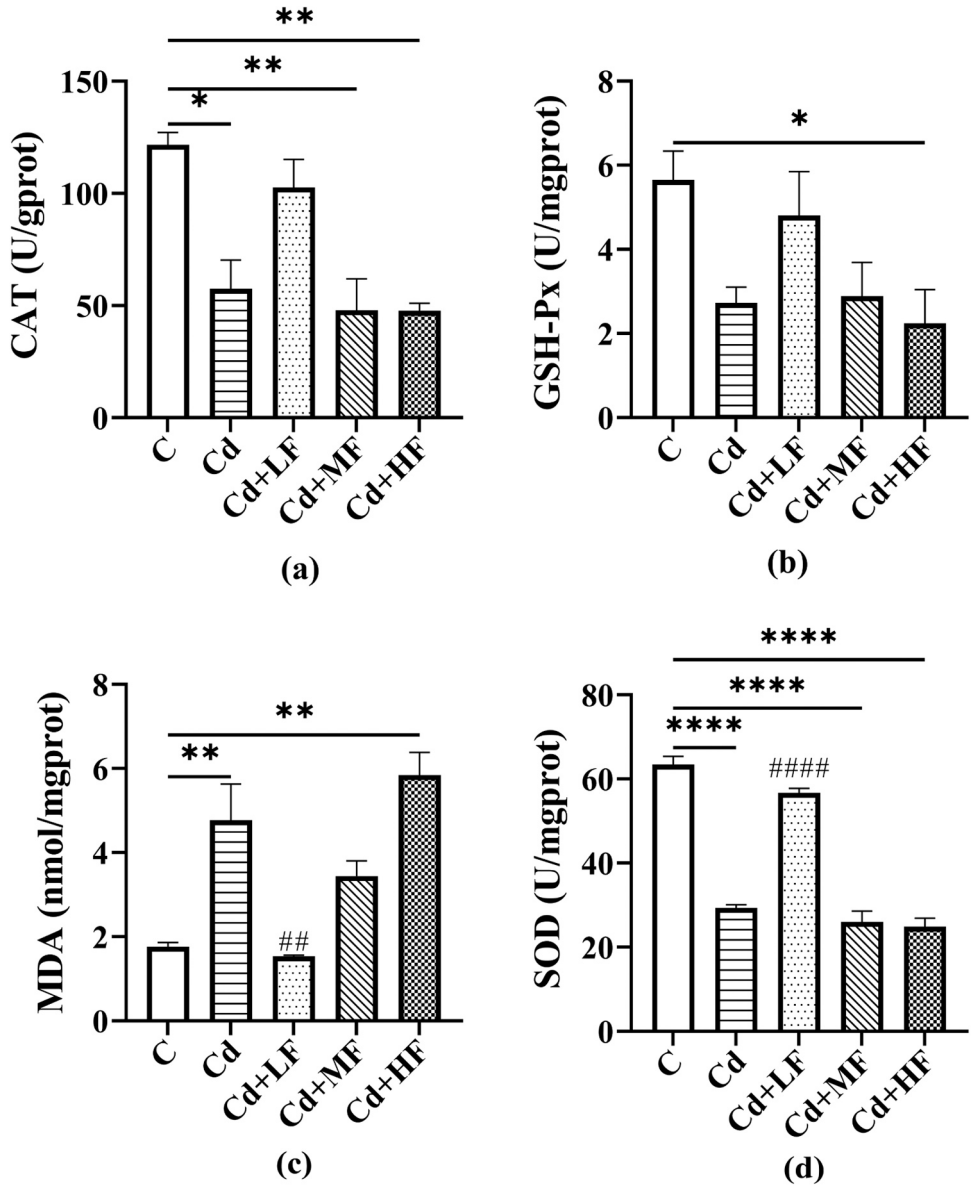

Fig. 1. Effect of F on Cd-mediated changes for (a) GSH-Px, (b) MDA, (c) CAT and (d) SOD in colon tissue of rats (n=3). *$p<$ 0.05, **$p<$ 0.01, ****$p<$0.0001 vs. control; $^{\#}p<$ 0.05, $^{\#\#\#}p<$ 0.001 vs. Cd group.

addition, the F accumulation in colon contents elevated in the Cd+LF, Cd+MF, and Cd+HF groups with a dose-dependent manner ($p<0.0001$) (Figure S1b). It indicated that F and Cd exposure alone can accumulate in the colon, while the ability to inhibit Cd accumulation was attenuated with increase in the dose of F.

3.2. Effects for F on Cd-produced oxidative stress of colon

Understanding the role of F in the oxidative damage caused by Cd, following indices were evaluated (Fig. 1). Compared to the C group, the MDA content was significantly elevated ($p<0.05$) (Fig. 1c) and the activities of CAT and SOD were significantly reduced ($p<0.05$; $p<0.0001$) (Fig. 1a and Fig. 1d). Compared to the Cd group, the activities of CAT,

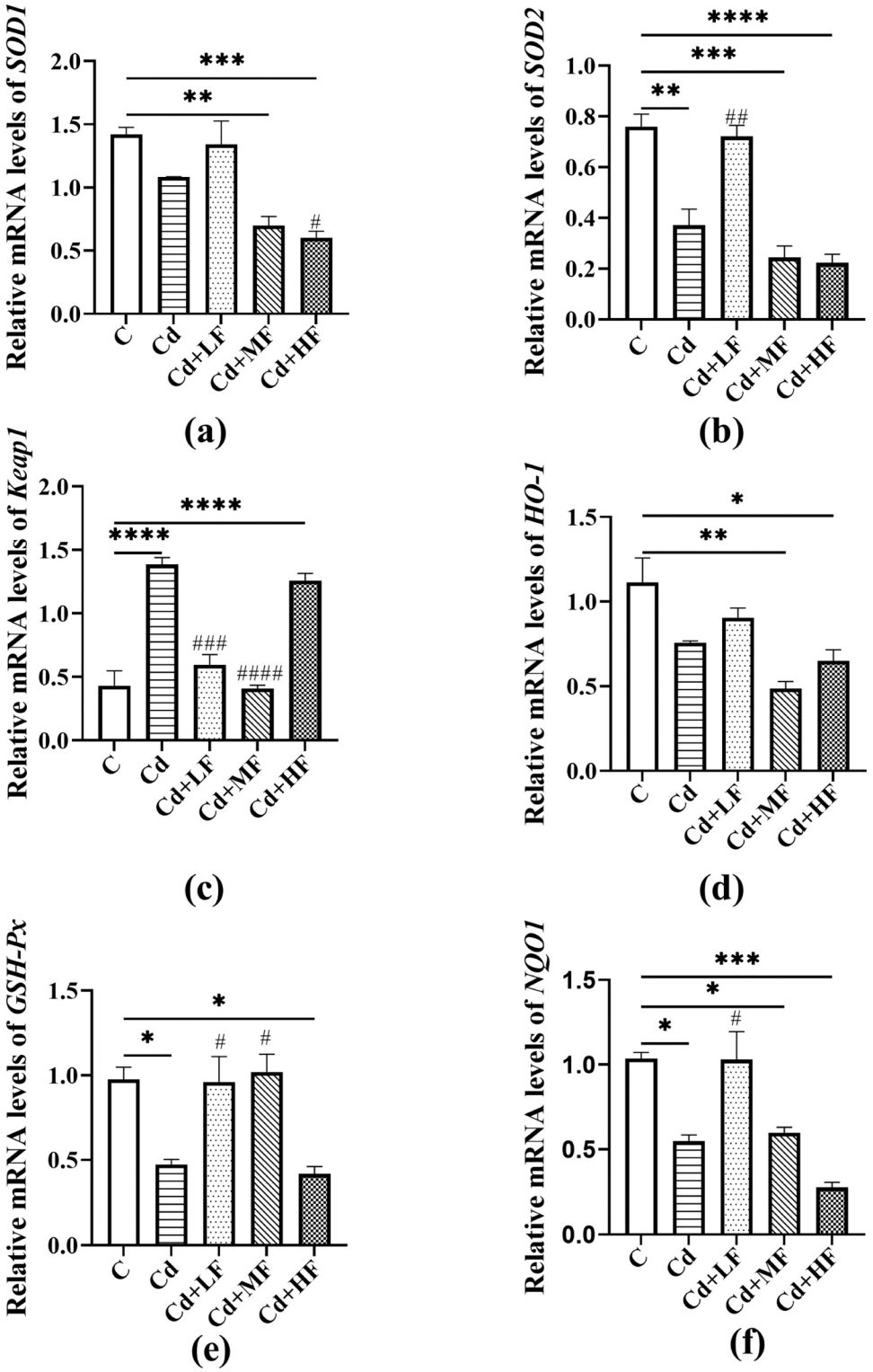

Fig. 2. Effect of F on Cd-induced the mRNA expression changes of (a) *Keap1*, (b) *HO-1*, (c) *NQO1*, (d) *GSH-Px*, (e) *SOD1*, and (f) *SOD2* in colon tissue of rats (n=3). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ vs. control; $^{\#}p< 0.05$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ vs. Cd group.

GSH-Px, and SOD in the Cd+LF group were enhanced 0.93, 1.76, and 1.78 times, respectively, and the MDA content reduced 0.67 times ($p<0.05$). Moreover, those results above in the Cd+HF group all showed opposite changes in relation to the Cd group.

In addition, the expression of *SOD2*, *GSH-Px,* and *NQO1* mRNA was significantly decreased by Cd treatment ($p<0.01$; $p<0.05$), while significantly elevated *Keap1* ($p<0.0001$) (Fig. 2). Compared to the Cd group, the results for *SOD2*, *GSH-Px,* and *NQO1* were significantly improved in the Cd+LF group ($p<0.05$; $p<0.01$; $p<0.05$), while the level of *Keap1* markedly reduced ($p<0.001$). Nevertheless, all genes in the Cd+HF group showed opposite trends to those that in the Cd+LF group. No statistical differences were revealed between the Cd and Cd+HF groups. In line with the changes of SOD, GSH-Px, CAT, and MDA, all data indicated that Cd exposure could trigger oxidative injury of colon in rats. However, it speculated that low F dosage might attenuate the intestinal oxidative stress by Cd produced, and a high F dosage should be inclined to aggravate that damages of colon in rats.

3.3. Effects of F on Cd-mediated intestinal permeability

3.3.1. Serum LPS levels

Compared with the C group, serum LPS levels were significantly intensified ($p<0.01$), indicating that individual Cd exposure might enhance the intestinal permeability in rats. Compared to the Cd group, LPS level was significantly reduced by 45.93 % ($p<0.05$) in Cd+LF group and increased by 12.70 % in Cd+HF group (Figure S2).

3.3.2. Relative expressions for Claudin-1, Occludin and ZO-1 of colon in rats

Claudin-1, *Occludin*, and *ZO-1* mRNA was determined by RT-qPCR (Fig. 3). Relative to C group, Cd treatment significantly reduced the relative expression of *ZO-1* and *Occludin* ($p<0.0001$, $p<0.001$). Relative to the Cd group, the levels of three genes were all up-regulated in the Cd+LF group, with the *ZO-1* expression increasing 2.09 times ($p<0.001$). In addition, the mRNA expressions of three indexes in the Cd+HF group were all down-regulated compared to the Cd group, decreasing by 35.46 %, 27.23 %, and 16.32 %, respectively.

In agreement with the results of LPS levels in the serum, the intestinal permeability in rats might be damaged via the Cd mediation. A low dose of F could inhibit the increase in intestinal permeability, while a much higher dose of F was more likely to increase intestinal permeability via Cd exposure.

3.4. Effects of F on Cd-induced inflammatory reaction of colon

Compared to the C group, Cd exposure significantly up-regulated the levels of IL-4 and IL-1β ($p<0.05$; $p<0.01$), and significantly down-regulated the IL-10 level ($p<0.01$) (Fig. 4). In the Cd+LF group, the levels of TNF-α, IL-1β, and IL-4 were suppressed and the IL-10 level was increased when relative to the Cd. However, the levels of TNF-α, IL-1β, and IL-4 were elevated in the Cd+HF group.

In addition, the mRNA expressions of *TNF-α*, *TLR-4*, *IL-1β*, and *IL-10* were further estimated (Fig. 5). Relative to the C group, Cd exposure increased *TNF-α*, *IL-1β* and *TLR-4* mRNA expression, and even *IL-10* was reduced by 50.51 %. Compared with the Cd group, the results for *TNF-α*, *IL-1β* ($p<0.05$) and *TLR-4* ($p<0.001$) were markedly inhibited in the Cd+LF treatment group. However, all of the genes in the Cd+HF group showed converse changes with an exception of *TNF-α*.

Collectively, Cd could trigger the intestinal inflammatory response in rats. Meanwhile, a low dosage of F could reverse the inflammatory injury, while a high dosage of F could co-enhance this damage via the Cd exposure.

3.5. Correlation analysis

To better characterize the interaction of F and Cd accumulation, as well as other related indexes of intestinal injury in rats, correlation analysis was performed (Figure S3). We found that F levels showed a significant correlation with serum LPS levels ($p<0.05$, r=0.90). The Cd concentration was well associated with the levels of IL-1β ($p<0.05$, r=0.85), IL-10 ($p<0.05$, r=0.87), IL-4 ($p<0.05$, r=0.87) and the relative expression of *ZO-1* ($p<0.05$, r=0.86), respectively. Furthermore, other correlations were happened in the pairwise comparisons, such as *IL-1β* mRNA and *Claudin-1* mRNA ($p<0.05$, r=0.86), and GSH-Px and T*NF-α* ($p<0.05$, r=0.90). Moreover, another indicators between oxidative stress, intestinal permeability, and inflammatory responses were also observed significant correlations.

4. Discussion

As two representative pollutants, literature have proved that Cd and/or F exposure generated harmful effects on organs and tissues (Luo et al.,

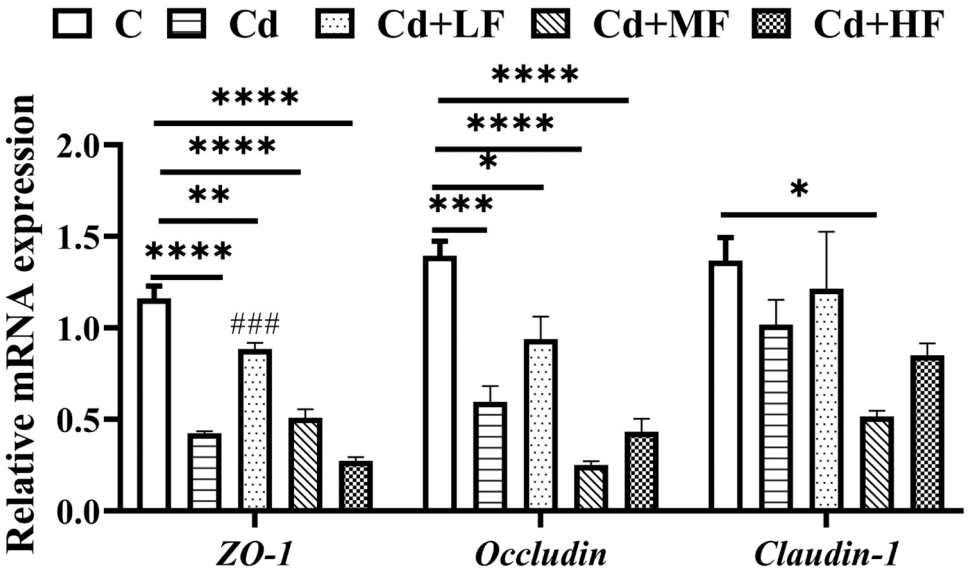

Fig. 3. Effect of F on the mRNA expression decreasing of (a) *ZO-1*, (b) *Occludin*, and (c) *Claudin-1* by Cd mediated (n=3). *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ vs. control; ###$p<0.001$ vs. Cd group.

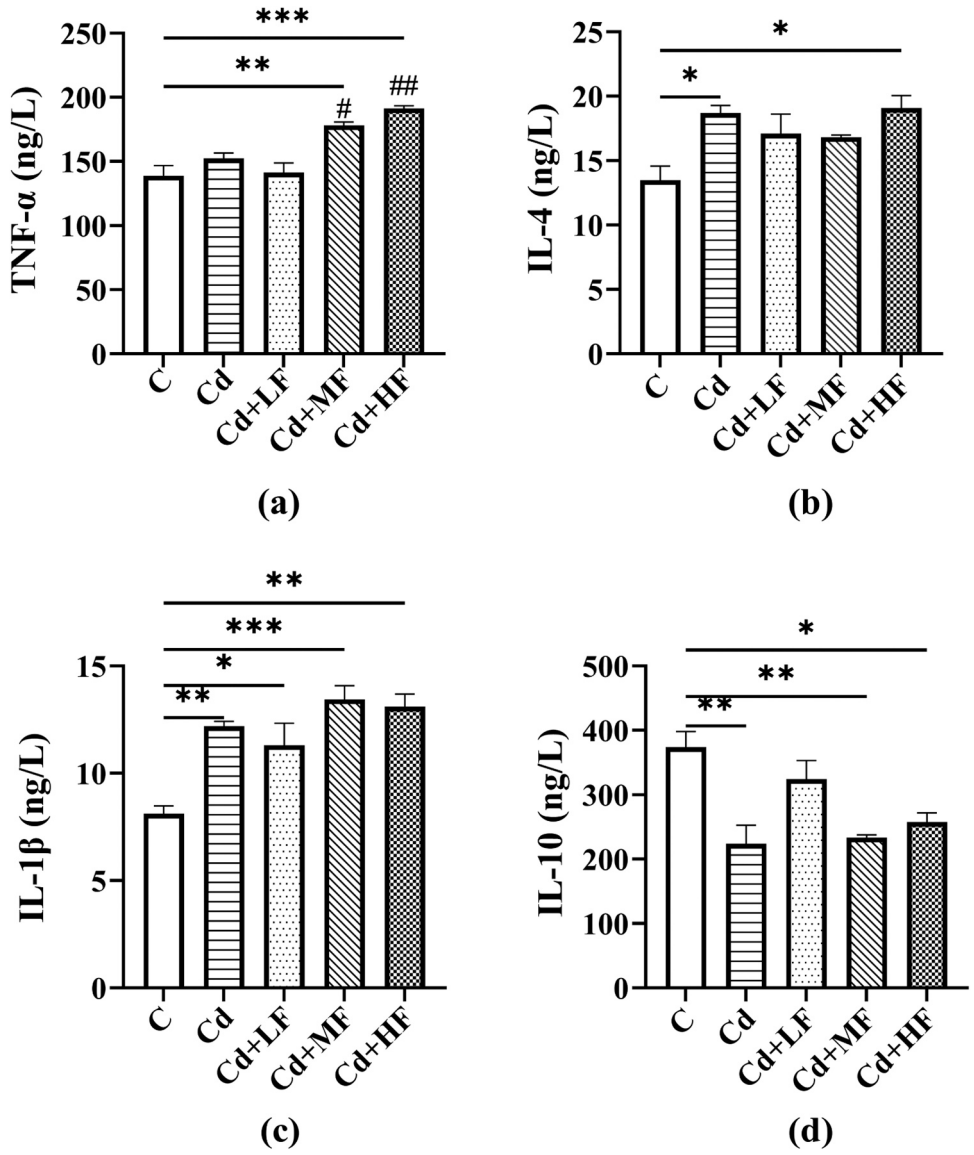

Fig. 4. Effects of F on Cd-mediated changes of (a) TNF-α, (b) IL-1β, (c) TLR-4, and (d) IL-10 (n=3). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ vs. Cd group.

2020; Kumar et al., 2021). Still, few studies were conducted to elucidate the toxic interaction of F and Cd co-exposed in the intestine of rats. For this purpose, the effects of different doses of F on Cd-induced intestinal barrier damage were monitored to define the toxic relationship between F and Cd in rats. As a consequence, we found that two-faced actions of F were observed in Cd-yielded oxidative stress, intestinal permeability increasing, and the inflammatory response of intestine in rats (Fig. 6).

The intestinal barrier is a critical factor in the limitation of Cd absorption, as previous study has demonstrated (Tinkov et al., 2018). However, prolonged accumulation of Cd compromises the integrity of the intestinal barrier leading to leaky gut. Wang et al. (2020) demonstrated that by oral administration, Cd can promote Cd accumulation and harm to the intestinal mucosa. Also, we found that Cd enriched in the intestine and disrupted intestinal barrier of rats compared with untreated group. The histopathological damages of colon in our previous study were in line with this conclusion (Li et al., 2023a, 2023b). It depicted that Cd treatment triggers incomplete intestinal mucosa with nuclei that were partially irregular and condensed, accompanied by a large infiltration of inflammatory cells. Intriguingly, the Cd levels were all statistically restrained by F. It might be attributed to the factor that F could easily bind with Cd to promote Cd discharge from the intestine (Johnston and Strobel, 2020). Also, other research claimed that F could hinder the uptake of calcium in the intestinal barrier (Sarić et al., 2002), but calcium could reduce the uptake of Cd (Spencer et al., 1969). Accordingly, we surmised that the Cd level for the intestine might be linked with the absorption of calcium. Noteworthy, further studies are needed to get a definite picture of the cumulative relationship between F and Cd.

One mechanism of Cd-related toxicity is well recognized as oxidative stress (Kumar, et al., 2019; Park et al., 2020). Excess Cd can restrain the activities of SOD, GSH-Px, and CAT, and elevate the level of MDA (Gupta et al., 2016; Chang et al., 2021). At the molecular level, Cd could trigger the induction of Nrf2 signaling pathway via significantly enhancing its downstream factors mRNA expression for *HO1* and *NQO1*, thereby resulting in oxidative disorder of intestine (Chen et al., 2019a, 2019b). These were in line with our findings. In this study, F at different doses presented diverse effects on oxidative stress by Cd-mediated. Specifically, low F (15 mg/kg) has the potential to attenuate the oxidative damage posed by Cd through increasing the expressions of antioxidant genes, while high F (75 mg/kg) could aggravate the intestinal oxidative damage. In a case of experiment, F reacted synergistically with Cd to exacerbate intestinal oxidative stress by increasing the levels

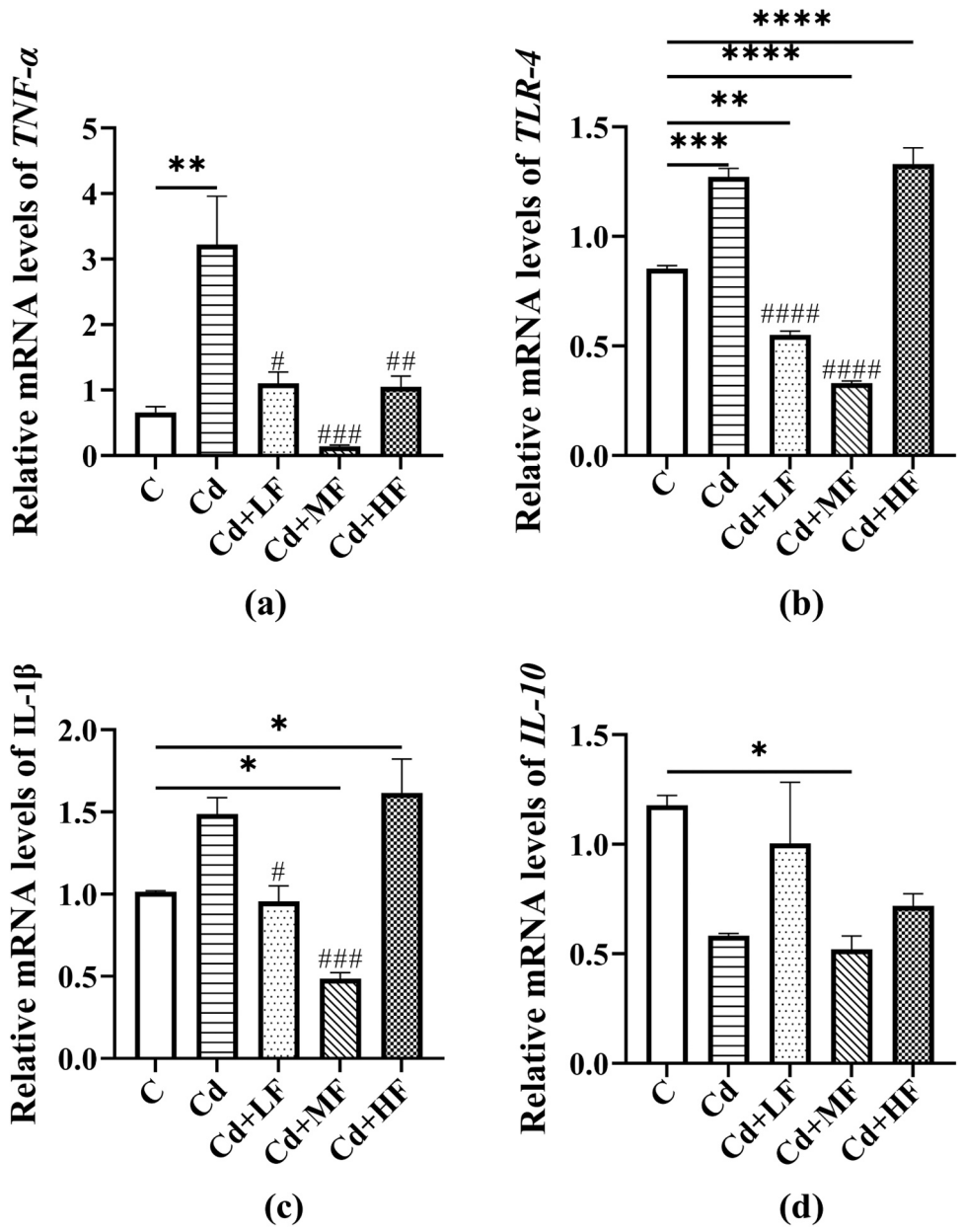

Fig. 5. Effect of F on Cd-induced the mRNA expressions of (a) *TNF-α*, (b) *IL-1β*, (c) *TLR-4*, (d) *IL-10* in colon of rats (n=3). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ vs. control; $^{\#}p<0.05$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ vs. Cd group.

of reactive oxygen species, and decreasing the activities of GSH-Px and CAT enzymes (Arab-Nozari et al., 2020). However, other reported that F enhances antioxidant enzyme activity and inhibits lipid peroxidation (Shayiq et al., 1986). Reasonably, we speculated that the influence of F on Cd induced oxidative stress might be dependent on the double threshold of F.

Oxidative stress can promote the degradation of tight junction proteins, leading to barrier dysfunction. The increased intestinal permeability observed in our study further illustrates this consequences of disrupted tight junction proteins. The serum LPS was identified as important indicators required for the characterization of intestinal permeability (Kakade et al., 2020; Suzuki, 2020). Cd exposure can interfere with the signal transduction pathway by increasing the LPS level, leading to the rupture of bacterial dissolution (Duizer et al., 1998; Hao et al., 2023). The cell model of Caco-2 showed that the Cd exposure could injure the adhesive junctions including ZO-1 and Claudin-1 (Fazeli et al., 2010). And Jiang et al. (2020) showed that Cd exposure down-regulated junctional proteins in vitro to destroyed intestinal barrier. Yu et al. (2020) showed that exposure of F could significantly attenuate the expression of ZO-1 and Occludin at gene and protein levels to result in the injury of intestinal barrier. As describe, our data were similar to these results. Though our research clarified the combined toxic effect of F and Cd in intestinal permeability for the first time, it cannot point the exact molecular mechanism about their combination. In the future, the mechanism of action of the combination of F and Cd needs to be further investigated at the molecular level using western blot, immunohistochemistry, immunofluorescence, etc, which will comprehensively strengthen our findings by correlating gene expression changes with these methods.

Experimental studies revealed that both F and Cd exert their toxic effects by inducing inflammation. IL-4 and IL-1β are regarded as pro-inflammatory cytokines secreted by the body that promotes inflammatory response, and IL-10 is a typical anti-inflammatory index designed to suppress inflammation (Vélez et al., 2019; Saxton et al., 2021). Cd

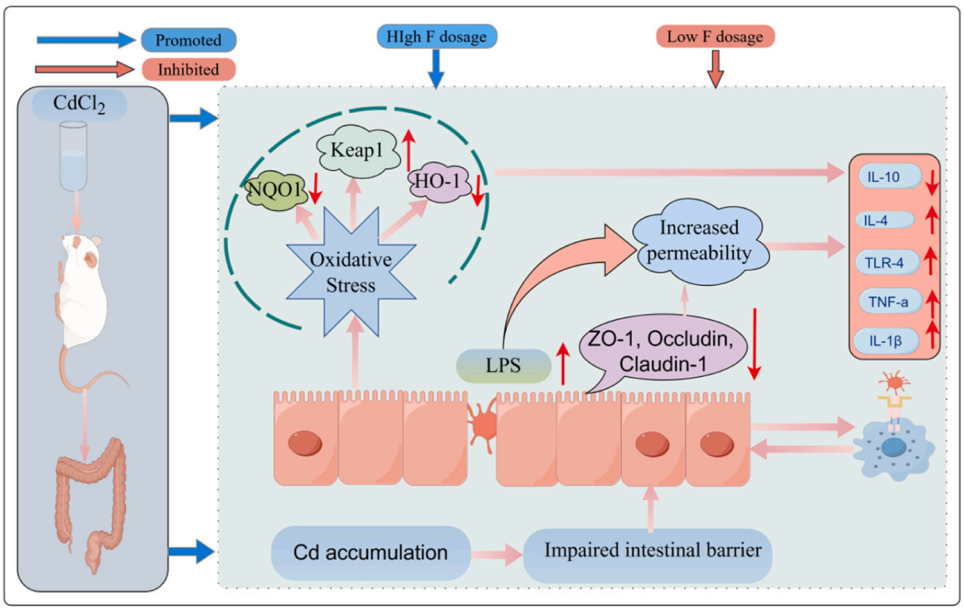

Fig. 6. The graphic showed two-sides effects of F on Cd induced intestinal damages of rats (By Figdraw: PAAYOa4c00).

exposure can produced TNF-α, IL-4 and IL-1β to enhance inflammatory response (Yang et al., 2021a, 2021b). Another study also observed a significant reduction in the increase of inflammatory mediators in male rat liver tissue in association with F exposure (Alhusaini et al., 2018). Notably, Arab-Nozari et al. (2020) pointed that combined exposure to F and Cd significantly up-regulated TNF-α and IL-1β inflammatory gene expressions to elevate the inflammatory damage in rats. In the present study, we found that Cd triggered intestinal inflammation with the increased levels of IL-4, TNF-a, IL-10, and IL-1β. Intriguingly, low levels of F were able to resist Cd and high levels of F enhanced Cd mediated intestinal inflammation in rats. Likewise, it can be assumed that the F and Cd interaction is largely determined by the dose of F.

Moreover, due to the complexity of the pathogenic mechanisms present in the intestinal environment, interactions among the intestine injuries were initially explored by the Pearson correlation analysis (Figure S3 and Fig. 6). The data can conclude that there are intricate correlations among the content of F and Cd, oxidative stress, intestinal permeability, and inflammation. Furthermore, the alterations of the gut microbiota are also considered in our previous study and evidenced closely associated with the intestinal barrier damage. Nevertheless, the complexity of microbial and gut environmental pathogens means that the current study cannot pinpoint the exact relationship and mechanism of action. Although the technique of 16S rDNA gene sequence analysis is widely used, it is not able to fully reveal the transcriptional and metabolic activities of the active micro-organisms in the gut. Therefore, to elucidate the mechanism of combined F and Cd intestinal injury, the molecular response to F and Cd intervention can be further investigated in the future by macrogenomics, macrotranscriptomics, and macrometabolomics comprehensively.

5. Conclusion

In conclusion, our study highlight that Cd injured intestinal barrier through mediating oxidative stress, intestinal permeability, and the inflammatory response in rats. However, F at specific doses has significant two-sided effects on Cd-induced intestinal barrier damage. Overall, we highlights that the potential beneficial or detrimental effect of F on Cd-mediated intestinal damages of rats mainly depends on F dosages. It extends our current knowledge and provides a valuable point of reference for the relationship between F and Cd. However, more exact molecular mechanisms underlying F and Cd have not been well understood and needed to be better elucidated in the future study.

CRediT authorship contribution statement

Zihao Zhou: Resources, Project administration. **Qinju Li:** Resources, Project administration. **Linchun Wang:** Methodology, Formal analysis, Conceptualization. **Dashuan Li:** Writing – review & editing, Writing – original draft, Software, Investigation. **Chaoxuan Liao:** Writing – original draft, Funding acquisition. **Yuhua Yang:** Methodology, Formal analysis, Conceptualization. **Jianzhong Cheng:** Validation, Funding acquisition. **Qinghai Zhang:** Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117030.

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