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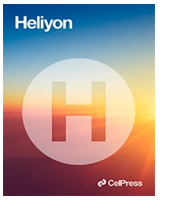

Research article

Protective effect of chitosan-modified rice porous starch loaded catechin on HT-29 cells exposed to lead ion

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ABSTRACT

Objective: To explore how chitosan-modified rice porous starch-loaded catechin (CT@RPS/CS) protects HT-29 cells exposed to lead ions. Method: The HT-29 cells were treated differently based on their grouping. The effect of CT@RPS/CS on lead-induced toxicity was evaluated using cell proliferation, apoptosis, oxidative stress index, and cytokine tests. Results: CT@RPS/CS did not affect the activity, cell apoptosis, oxidative stress level, and related cytokines of HT-29 cells. After exposure to lead, CT@RPS/CS has the potential to enhance cellular activity, minimize apoptosis, and decrease the level of oxidative stress. Discussion: CT@RPS/CS not only has no toxicity to cells but also adsorbs lead ions, which protects cells.

1. Introduction

Lead (Pb) is a widespread industrial pollutant and one of the ten chemicals recognized as causing major public health concerns [1]. Although lead pollution is decreasing yearly in China, it cannot be ignored in food [2,3]. The lead form currently in use has a direct impact on how much lead organisms absorb and how toxic the lead is to them. Exposure to lead pollution from various sources, such as contaminated soil and water, can have a significant impact on human health [4]. High-dose or long-term low-dose lead poisoning can lead to decreased immunity, affect the functions of human nervous, cardiovascular, skeletal, and reproductive systems, diseases of various organs, or even cancer [5–7]. Centers for Disease Control and Prevention of the United States (CDC) believes that there is no safe threshold for blood Pb concentration, and it is recommended that children's blood Pb concentration $\geq$100 μg/L is lead poisoning.

Rice porous starch (RPS) is generally prepared from rice starch using the enzymatic method, chemical method, or chemical method combined with the enzymatic method [8]. It has the advantages of small particle size, strong adsorption, easy digestion, and low allergens. Chitosan (CS), a natural cationic polysaccharide, is a non-toxic substance with antioxidant activity. CS is a potent antioxidant, and it can prevent lipid oxidation in biological systems by scavenging free radicals and inhibiting ROS production [9–11]. Catechin (CT) is rich in phenolic hydroxyl groups, has a potent free radical scavenging ability, and has a variety of biological activities. Previous studies have shown that catechin can significantly inhibit DNA oxidative damage [12], reduce the occurrence of inflammation [13,14], have a positive effect on sarcoplasmic reticulum Ca^{2+} [15], effectively prevent neuroinflammation [16] and reduce the occurrence of Alzheimer's disease and Parkinson's syndrome [17]. Meanwhile, catechins have good adsorption on heavy metals, such as lead and cadmium [18,19]. Catechin can protect the activity of HepG2 cells exposed to lead and inhibit lipid peroxidation [20].

There were few application studies on chitosan-modified rice porous starch-loaded catechin in food. Based on previous

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experimental research [21], it is found that CT@RPS/CS has a strong lead adsorption capacity. The cell HT-29 was used to establish an intestinal cell model to study whether CT@RPS/CS alleviates the cytotoxicity caused by lead exposure and explore whether CT@RPS/CS has a protective effect on HT-29 cells exposed to lead.

2. Materials and methods

2.1. Cell culture

The HT-29 Cell line is an excellent model to study the different parameters in some research [22–24]. HT-29 cells were cultured in RPMI-1640 complete medium at 37 °C in a 5 % CO2 incubator. The complete medium contained 10 % fetal bovine serum (Thermo Fisher biochemical products (Beijing) Co., Ltd), 1 % Penicillin/Streptomycin (Beyotime Biotechnology, China), and RPMI-1640 incomplete medium (HyClone company, USA); The cell culture medium was changed every 2–3 days. The cells were detached with 0.25 % trypsin (including 0.02 % EDTA) when the cell adhesion rate reached 80–90 %. Then, the cells were sub-cultured in 1:3 or 1:2. The cells were cultured in the logarithmic growth phase and selected in the following experiment. HT-29 cells were treated according to groups and inoculated into 96 well plates (1×10^5 cells/well), cultured for 12–24 h until the cells wholly adhered to the wall (observed by inverted microscope).

2.2. Chemicals and reagents

Lead acetate (AR, Pb(C2H302)2·3H20)) was purchased from Sinopharm Chemical Reagent Co., Ltd. Chitosan (degree of deacetylation 95 %, viscosity 100–200 mPas) was purchased from Shanghai Aladdin Chemical Reagent Co., Ltd. (China). Epicatechin gallate (EGCG, HPLC 98 %) was purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA). Milli-Q water was obtained from the Millipore Bedford purification system (MA, USA).

2.3. The preparation of porous chitosan-modified starch preparation

The following steps were taken in order to prepare RPS/CS powder [25]:1) 1 g of CS was dissolved in 400 mL of acetic acid solution (2 g per liter) and then mixed using magnetic stirring at 20 °C for 30 min 2) 3 g of RPS were evenly dispersed in the CS solution to create an RPS/CS dispersion with a CS concentration of 25 % (w/W). 3) After allowing the solution to precipitate for 24 h naturally, the supernatant was removed, and the precipitation was freeze-dried at −50 °C for 24 h to prevent gelatinization. Finally, the powder sample was heat-treated in an oven at 130 °C for 4 h and then cooled to room temperature. The resulting RPS/CS powder was collected through a 200-mesh sieve.

2.4. Experimental group design

The experiment involved using T-29 cells, which were divided into four groups. The groups were as follows: Control group, Only Pb (II) group(G-Pb), Only CT@RPS/CS group (G-C@R/C), and Pb (II) and CT@RPS/CS group (G-Pb + C@R/C). The following steps were taken for each group, as shown in Table 1.

(1) Control group: HT-29 cells were cultured in RPMI-1640 complete medium at 37 °C in a 5 % CO_2 incubator for 24 h.
(2) G-Pb: HT-29 cells were cultured in RPMI-1640 complete medium, containing 8 mM $(CH3COO)_2Pb{\cdot}3H_2O$, at 37 °C in a 5 % CO_2 incubator for 24 h for the various experiments.
(3) G-C@R/C: HT-29 cells were cultured in RPMI-1640 complete medium at 37 °C in a 5 % CO_2 incubator for 24 h. The complete medium containing 4 mg/mL CT@RPS/CS was used in the various experiments.
(4) G-Pb + C@R/C in Intervention group: HT-29 cells were cultured in RPMI-1640 complete medium at 37 °C in a 5 % CO_2 incubator for 24 h. The complete medium containing 8 mM $(CH3COO)_2Pb{\cdot}3H_2O$ and 4 mg/mL CT@RPS/CS was used in the various experiments.
(5) G-Pb + C@R/C in Therapy group: HT-29 cells were cultured in RPMI-1640 complete medium (containing 8 mM (CH3COO)2 Pb·3H2O) at 37 °C in 5 % CO_2 incubator for 24 h and then washed twice with the complete medium. Finally, the cells were cultured in RPMI-1640 complete medium (containing 4 mg/mL CT@RPS/CS) at 37 °C in a 5 % CO_2 incubator for 24 h.

Table 1
Experimental group design.

	Intervention group	Therapy group	Prevention group
Control group	cells were cultured without treated for 48 h		
G-Pb	cells were cultured with Pb (II) and treated for 48 h		
G-C@R/C	cells were cultured with CT@RPS/CS and treated for 48 h		
G-Pb + C@R/C	without treated for 24 h.	with Pb (II) treated for 24 h	with CT@RPS/CS treated for 24 h
	Pb (II) + CT@RPS/CS treated for 24 h	with CT@RPS/CS treated for 24 h	with Pb (II) treated for 24 h

(6) G-Pb + C@R/C in Prevention group: HT-29 cells were cultured in RPMI-1640 complete medium (containing 4 mg/mL CT@RPS/CS) at 37 °C in 5 % CO_2 incubator for 24 h and then washed twice with the complete medium. Finally, the cells were cultured in RPMI-1640 complete medium (containing 8 mM (CH3COO)2 Pb·3H2O) at 37 °C in a 5 % CO_2 incubator for 24 h.

2.5. Determination of cell proliferation activity

According to the manufacturer's instructions, the cell proliferation activity was assessed using the MTT assay kit (Beyotime Biotechnology, China). Briefly, the cells were cultured in 96-well in each experiment group and then incubated with 10 μL MTT solution (5 mg/mL) at 37 °C in an incubator for 4 h. They were then dissolved in 100 μL Formazan solution in an incubator for 4 h. Cell proliferation activity was determined by measuring the absorbance at 570 nm. This was done by using the measured OD value as a basis for calculation: a higher OD value indicates stronger cell activity. In comparison, a lower OD value indicates smaller cell activity.

2.6. Determination of cell apoptotic

According to the manufacturer's instructions, the cell apoptosis was assessed using an annexin V-FITC apoptosis detection kit (Beyotime Biotechnology, China). Each experiment group had 6-well cultured cells.1.0 × 10^5 suspended cells were taken after centrifuging (1000 g, 5 min). Discard the supernatant, add 195 μL annexin FITC binding solution, gently resuspend the cells, and then gently mix 5 μL Annexin FITC. Finally, add 10 μL PI, gently mixed and incubated at room temperature (20–25 °C) in the dark for 10–20 min, detected by flow cytometry immediately. If it cannot be detected in time, put it in an ice bath for a short time, store it in the dark with aluminum foil, and get it on the machine for detection as soon as possible.

2.7. Determination of reactive oxygen species

According to the manufacturer's instructions, ROS was assessed by a biochemical kit (Beyotime Biotechnology, China). The ROS probe was detected in the cells by immunofluorescence using a Laser confocal microscope (LSM880+Airyscan, Zeiss, Germany). LSM 800 provides high sensitivity for detecting weak fluorescent signals with low laser illumination and rapid frame rates. The system has a special ability to take highly detailed images of samples using spectral imaging and unmixing technology. It can also remove the unwanted autofluorescence background from low signals, which can often get in the way of important details. This feature is especially helpful when samples have a low signal-to-noise ratio. Researchers can use this system to get clear and accurate images.

2.8. Determination of SOD and MDA

Superoxide dismutase (SOD) is an antioxidant enzyme that can scavenge the superoxide anion. SOD has specificity and protects cells from free radical damage, which was assessed using a biochemical kit (Nanjing Jiancheng Institute of Bioengineering, China). The visible spectrophotometer measured the absorbance and calculated the SOD activity value according to formula (1).

$$A(SOD) = \frac{OD^{C}_{532} - OD^{S}_{532}}{OD^{C}_{532}} \div 50\% \times n_1 \times n_2 \tag{1}$$

A(SOD) represents the total SOD activity value (U/ml), OD^{C}_{532} and OD^{S}_{532} represents the OD value of the control group and the determination group at wavelength 532 nm, n_1 is the dilution multiple of the reaction system, n_2 represents the dilution multiple before the sample test.

Malondialdehyde (MDA) is one of the lipid peroxides produced by oxygen free radicals applying to the polyunsaturated fatty acids in biofilm, which can cause cell damage. According to the manufacturer's instructions, MDA was assessed by a biochemical kit (Nanjing Jiancheng Institute of Bioengineering, China). The MDA content value was calculated according to formula (2)

$$c(MDA) = \frac{OD^{S}_{532} - OD^{C}_{532}}{OD^{ST}_{532} - OD^{B}_{532}} \times 10\% \times n \tag{2}$$

$c(MDA)$ represents the content of MDA in the supernatant, S represents the OD value of the determination group, C represents the OD value of the control group, ST represents the OD value of the standard solution, and B represents the OD value of the blank control group, all measured at wavelength 532 nm. n represents the dilution ratio of the sample before the test. 10 represents the standard's concentration (the standard's concentration in this study is 10 nM).

2.9. Determination of cytokine content

According to the manufacturer's instructions, the ELISA kit assessed the cytokine content (Beyotime Biotechnology, China). Briefly, the cell supernatant was collected and temporarily stored in the refrigerator at - 20 °C, finally determining it.

2.10. Statistical analysis

This experiment expresses all experimental data by mean ± standard error (mean ± SD). At least three parallel samples are set in

each experiment. Results were analyzed by one-way ANOVA analysis and *t*-test. $P < 0.05$ is considered a statistical difference, and $P < 0.01$ is a significant difference in data comparison. PASW statistics version 18 for Windows software was used to analyze all experimental data processing.

3. Results

3.1. Effect of Pb(□) exposure on HT-29 cells

Compared with the control group, the cell survival rate was reduced under low-concentration Pb(□) exposure (0.5 mM and 1 mM), but the difference was not statistically significant ($p > 0.05$). With the increase of Pb (□) concentration, the survival rate of HT-29 cells decreased continuously when the survival rate was only 18.81 % under 10 mM Pb (□) exposure. Under 8 mM Pb (□) exposure, the survival rate was 47.35 %, significantly different from the blank control and 10 mM Pb (□) exposure. The result is consistent with the literature report [26], which reflects that the concentration of 8 mM Pb (□) can produce relatively moderate cytotoxicity (Fig. 1).

3.2. Protective effect of CT@RPS/CS from Pb (□) exposure to HT-29 cells

From Fig. 2, The HT-29 cells were exposed to 8 mM Pb (□) at a low concentration of CT@RPS/CS had a fragile protective effect on the survival of cells, such as when the concentration of CT@RPS/CS was 0.4 mg/mL, the survival of cells was only 47.91 % which was significantly lower than that of the control group ($P < 0.05$). When the concentration of CT@RPS/CS continues to increase to 10 mg/mL and 20 mg/mL, the survival of cells is like that of when the concentration of CT@RPS/CS is 8 mg/mL. Nevertheless, the survival of cells was similar between the concentration of CT@RPS/CS being 8 mg/mL and the concentration of CT@RPS/CS being 4 mg/mL. So, at last, 4 mg/mL of CT@RPS/CS was selected for the following further study.

3.3. The activity effect of CT@RPS/CS on Pb (□) exposed HT-29 cells

The effect of CT@RPS/CS on the survival rate of HT-29 cells under 8 Mm Pb (□) exposure is shown in Fig. 3. Fig. 3 A, 3B, and 3C represent the intervention, therapy, and prevention groups. In the study, the survival rate of HT-29 cells in G-Pb was significantly lower than that in the Ctrl group and G- C@R/C in the intervention, therapy, and prevention groups. Whether in the intervention group, the therapy group, or the prevention group, the results reflected that the survival rate of HT-29 cells is higher than that of HT-29 cells in the G-Pb group, the survival rate of G- C@R/C + Pb group, 88.91 %, 79.70 %, and 90.55 % respectively.

3.4. The apoptotic effect of CT@RPS/CS on Pb (□) exposed HT-29 cells

The flow cytometry diagram in Fig. 4 contains four quadrants [27]: Q1 represents necrotic cells, Q2 represents a non-viable apoptotic cell, Q3 represents a viable apoptotic cell, and Q4 represents living cells—data analysis of each in Fig. 5. Fig. 4 shows that the number of cells in Q2 and Q3 in the G-Pb group is significant in the Ctrl group in the intervention, therapy, or prevention groups. The number of cells in the Q4 quadrant is lower than that in the Ctrl group, In the G-C@R/C group, the number of cells in Q2, Q3, and Q4 quadrants were like that in the Ctrl group, which was no significant difference in the corresponding quadrant comparison, and viable apoptotic rate and non-viable apoptotic rate were much lower than those in G-Pb group. Fig. 5 showed the data in the G-Pb + C@R/C group, the order of living cell ratio was intervention group > prevention group > therapy group; The order of viable

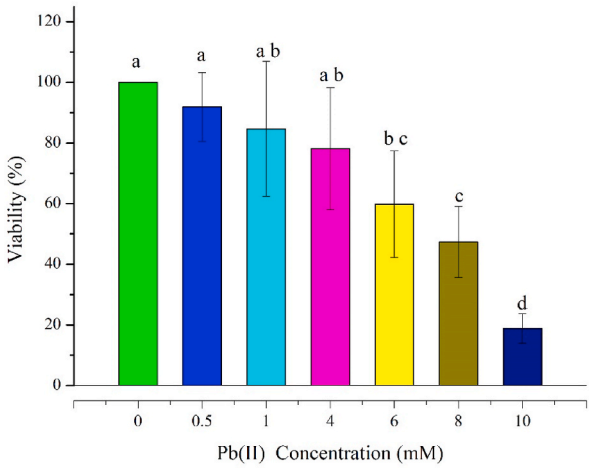

Fig. 1. Effects of Pb (□) exposure concentrations on the viability of HT-29 cells. Note: Viability represents the rate of the treatment group vs. the Ctrl group. a, b, c, and d represent statistical differences in intra-group comparisons, respectively.

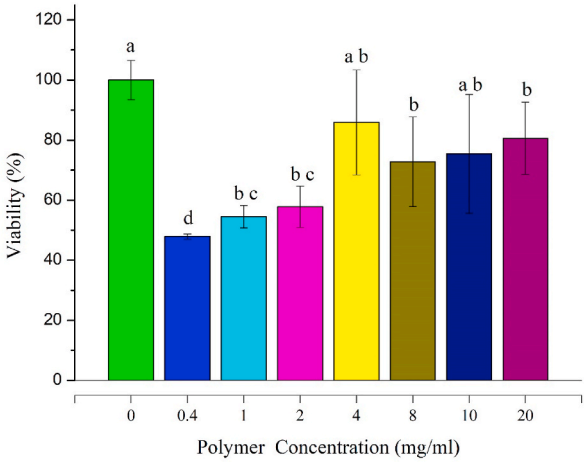

Fig. 2. Effects of the viability of HT-29 cells by different concentrations of CT@RPS/CS. Note: Viability represents the rate of the treatment group vs. the Ctrl group. A, b, and c represent statistical differences in intra-group comparisons, respectively.

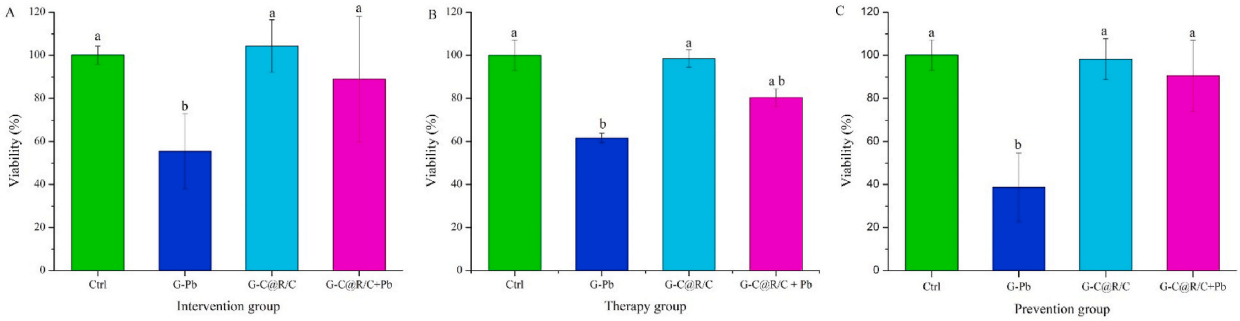

Fig. 3. Cell viability of HT-29 cells in intervention, therapy, and prevention group. Note: Viability represents the rate of the treatment group vs. the Ctrl group. A, B, and C represent intervention, therapy, and prevention groups. a and b represent statistical differences in intra-group comparisons, respectively.

apoptotic cell ratio was therapy group > prevention group > intervention group, and the order of non-viable apoptotic cell ratio was prevention group > intervention group > therapy group.

3.5. The content changes of reactive oxygen species (ROS) in HT-29 cells

The DCFH-DA fluorescence probe can observe the content of ROS in HT-29 cells, and the number and fluorescence intensity of ROS at 488 nm can reflect the level of ROS. Under the observation of the laser confocal microscope, the ROS content of HT-29 cells in different treatment groups is shown in Fig. 6. The green fluorescent represented the ROS level. Whether in the intervention, therapy, or prevention groups, the green fluorescence point is evident in the G-Pb group, which reflects high ROS level content. The content of ROS in G-C@R/C (Fig. 6C(I), C(T), and C(P)) was slightly higher than that in the Ctrl group (Fig. 6 A(I), A(T), and A(P)) but significantly lower than that in the G-Pb group (Fig. 6 B(I), B(T), and B(P)). The number of fluorescent cells and fluorescence intensity in the visual field decreased to varying degrees (Fig. 6 D(I), D(T), and D(P)), indicating that CT@RPS/CS can reduce the content of ROS. In the treatment group or prevention group, the number of fluorescent cells in the visual field decreased significantly, reflecting that CT@RPS/CS can reduce the content of ROS, but it is limited to protective and preventive (Fig. 6D (T) and 6D (P)).

Fig. 7A, B, and 7C shows that in the intervention group, therapy group, and prevention group, the SOD activity values in the G-Pb group are lower than that in the Ctrl group, with differences in comparison. In the G-C@R/C group, SOD activity was higher than that in the Ctrl group, with differences in comparison. In the intervention and therapy groups, the SOD activity value was no significant difference between G-Pb + C@R/C and G-Pb group. Still, the activity value in the prevention group was slightly higher than that in the Ctrl group and higher than in the G-Pb group, with differences in comparison.

3.6. Detection of SOD and MDA in HT-29 cells

Fig. 7D, E, and 7F show that in the intervention, therapy, and prevention groups, the MDA value in the G-Pb group is higher than

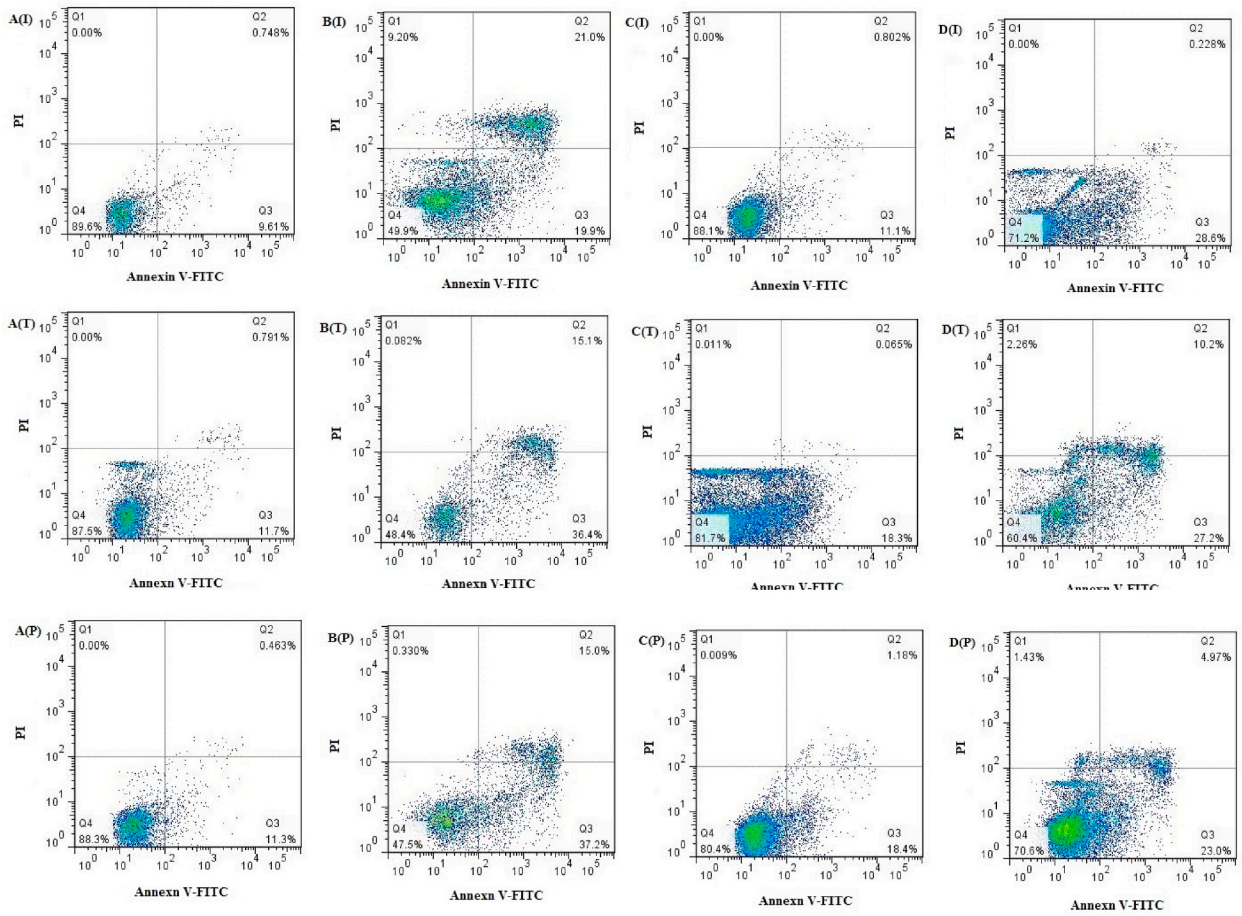

Fig. 4. Flow cytometry of HT-29 cells apoptosis in each group. Note: A, B, C, and D represent Ctrl, G-Pb, G-C@R/C, and G-Pb + C@R/C, respectively. I, T, and P represent the intervention, therapy, and prevention group, respectively.

that in the Ctrl group, with a difference in comparison. In the G-C@R/C group, MDA values were almost the same as those in the Ctrl group, and there was no significant difference between G-C@R/C and Ctrl groups. In G-Pb + C@R/C, the MDA values were lower than those in group G-Pb and slightly higher than those in the Ctrl and G- C@R/C groups.

3.7. Detection of secreted cytokines

The distribution of cytokines in each group is shown in Fig. 8. Whether in the intervention group, the therapy group, or the prevention group, the cytokine concentration in the G-Pb group is the highest than other groups, with the difference in comparison, except for TNF concentration in the therapy group. The cytokines concentration in the G- C@R/C group is similar to or slightly lower than that in the Ctrl group, with no difference in comparison. In the intervention group, the immune factor (IL-1, IL-6, IL-8, and IL-10) of G-C@R/C was slightly lower than that of the Ctrl group. The TNF of G-C@R/C is like that of the Ctrl group and slightly higher than that of the G-Pb + C@R/C Group. The comparative difference is shown in Fig. 8 (A1, A2, A3, A4, A5). In the therapy group, the immune factor (IL-1, IL-6, IL-8, IL-10, and TNF) of G-C@R/C was slightly lower than that of the Ctrl group G-Pb + C@R/C Group, with no difference in comparison. The comparative difference is shown in Fig. 8 (B1, B2, B3, B4, and B5).In the prevention group, the immune factor (IL-1, IL-6, IL-8, IL-10, and TNF) of G-C@R/C was like or slightly lower than that of the Ctrl group, Except IL-10; there was no significant difference compared with the Ctrl group, as shown in Fig. 8 (C1, C2, C3, C4, and C5).

4. Discussion

As far as we know, oral lead can have a series of adverse effects on the human body, and the intestine is the major organ that directly contacts and absorbs lead. Previous studies have shown that lead toxicity was one of the essential mechanisms that caused oxidative stress [28]. Consistent with our results, the production of reactive oxygen species and oxidative stress was induced by lead in PC12 cells [20]. Moreover, lead-induced cell death depends on the level of reactive oxygen species [29], and the mitochondria were damaged by

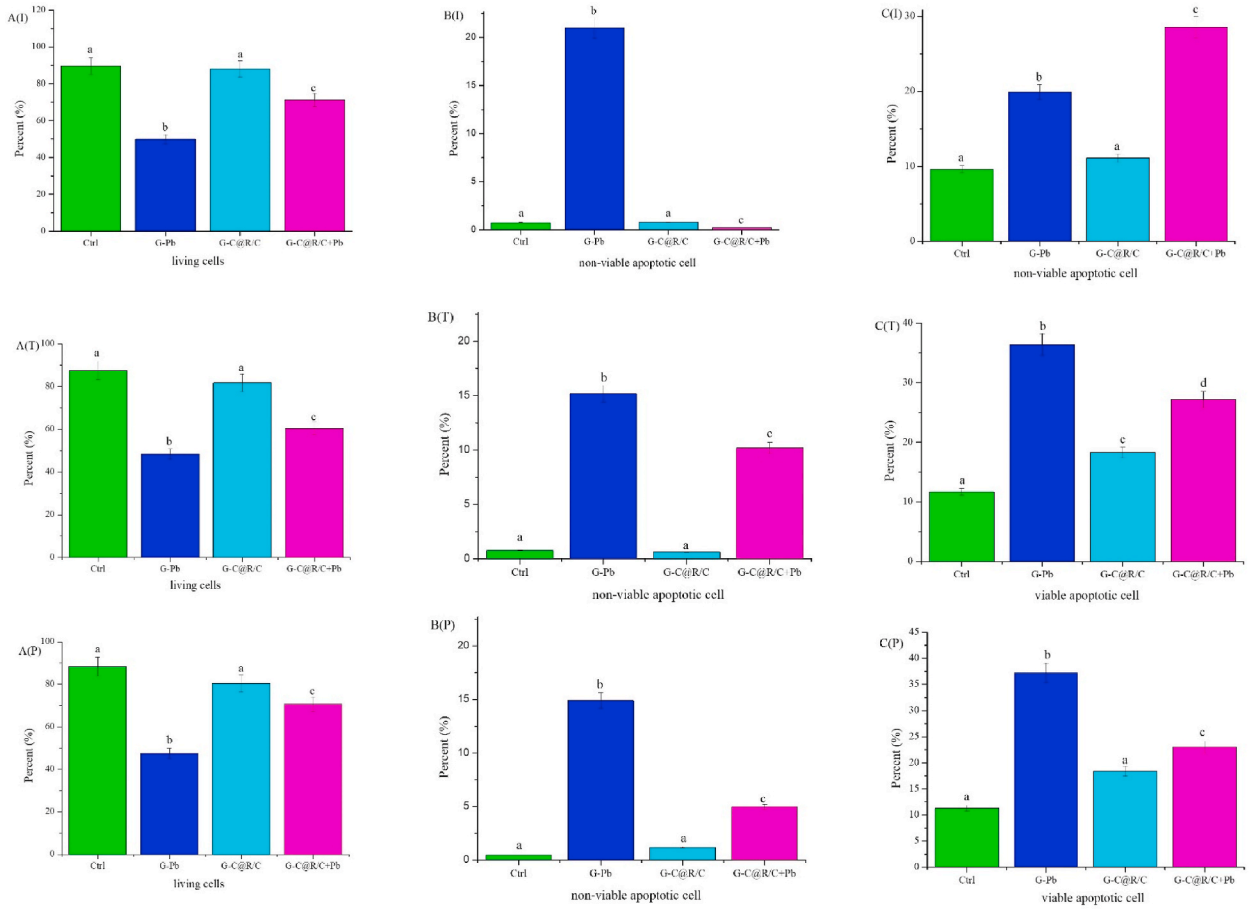

Fig. 5. Data analysis of HT-29 cell apoptosis in each group. Note: A, B, and C represent the living cell, non-viable apoptotic cell, and viable apoptotic cell, respectively. I, T, and P represent intervention, therapy, and prevention groups. a, b, c, and d represent statistical differences in intra-group comparisons, respectively.

the excessive production of ROS, which would lead to cell dysfunction [30]. In addition, a previous study showed that the nuclei of intestinal cells, which may be detected by transmission electron microscopy, were a powerfully negative effect of lead [31].

In this study, 8 mM Pb (II) was used in vitro, leading to 50 % mortality and elevated levels of reactive oxygen species, which is consistent with the literature [26]. The results (Fig. 1) showed that the activity of HT-29 cells was less affected by a low concentration of Pb (II) exposure. When the concentration of Pb (II) was low (0.5 mM and 1 mM), the survival rate of HT-29 cells decreased. When exposed to 8 mM Pb (II), the survival rate of HT-29 cells was only 47.35 % of the control group. When exposed to 10 mM Pb (II), the survival rate of HT-29 cells was only 18.81 %, which was not conducive to the later experiment. Therefore, 8 mM was selected as the moderate exposure concentration, consistent with the literature [26]. The results (Fig. 2) showed a little protective effect on lead-exposed cells at low concentrations of CT@RPS/CS. When CT@RPS/CS concentration reaches 4 mg/mL, there is no significant difference in the survival rate of HT-29 cells under 8 mM lead exposure compared with the control group. With the increase of CT@RPS/CS concentration, the survival rate of lead-exposed cells does not increase significantly, suggesting that CT@RPS/CS of 4 mg/ml can be used as the experimental concentration for later experiments.

Whether in the intervention group, treatment group, or prevention group, CT@RPS/CS can improve the survival rate of HT-29 cells after lead exposure; that is, it has a protective effect. In addition, when only CT@RPS/CS was used, there was no significant difference between the survival rate of HT29 cells and that of the control group, suggesting that CT@RPS/CS had no cytotoxicity. Through the apoptosis experiment, whether in the intervention group, treatment group, or prevention group, the apoptosis rate of the G-C@R/C group was significantly lower than that of the G-Pb group. The comparison difference was statistically significant, almost the same as that of the Ctrl group, which also showed that CT@RPS/CS had no toxic effect on cells (as shown in Fig. 4). After treatment with CT@RPS/CS, the apoptosis rate decreased significantly. In the G-Pb + C@R/C group, the apoptosis rates of the intervention group, treatment group, and prevention group were 29.92 %, 37.4 %, and 28.63 %, respectively, which was significantly lower than that of the G-Pb group, suggesting that CT@RPS/CS treatment can alleviate the damage of Pb (II) exposure to cells to a certain extent, as shown in Fig. 5.

Many previous studies have shown that Pb(II) exposure can lead to oxidative stress damage of kidney cells, liver cells, nerve cells,

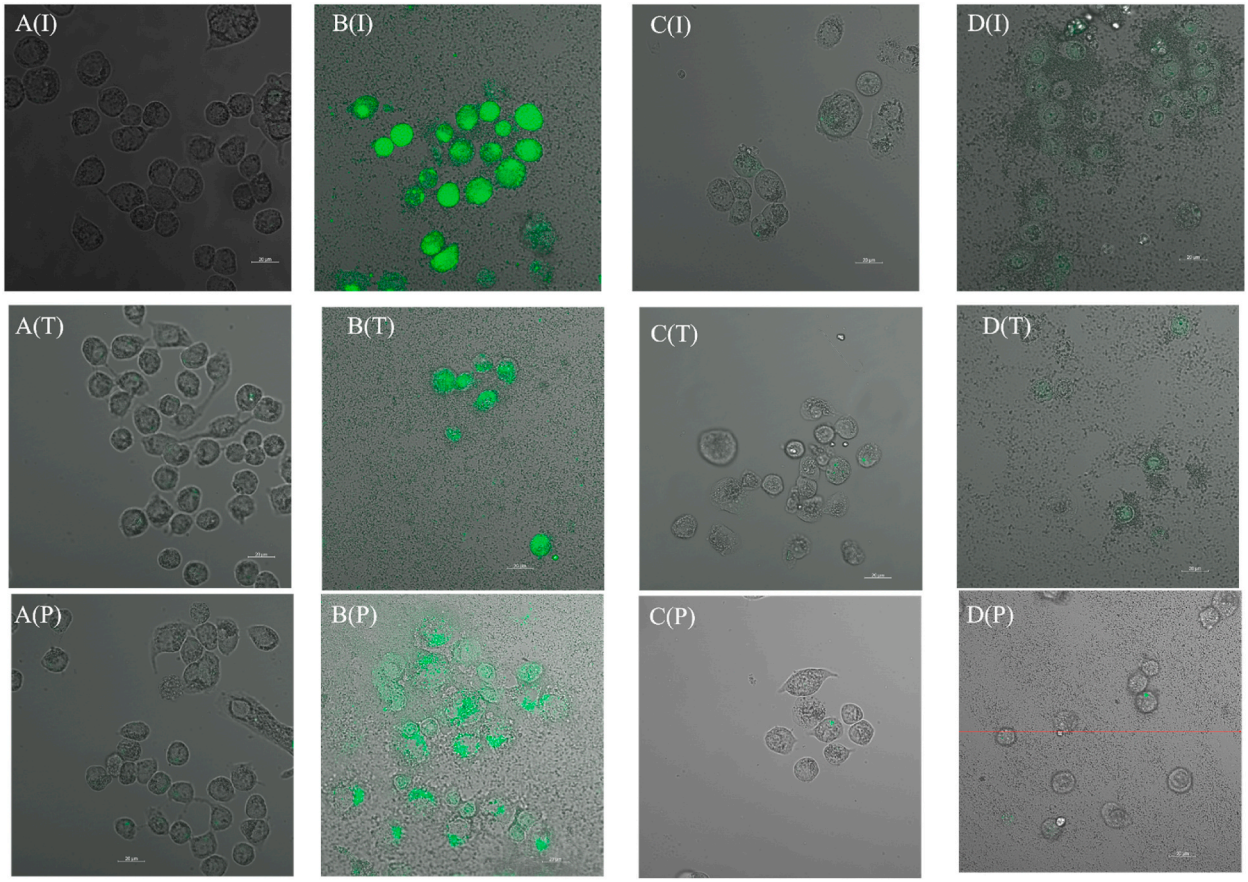

Fig. 6. Laser confocal image of HT-29 cells in each group. Note: A, B, C, and D represent Ctrl, G-Pb, G-C@R/C, and G-Pb + C@R/C, respectively. I, T, and P represent intervention, therapy, and prevention groups.

and intestinal cells, especially the rise of ROS level, damaging biological macromolecules such as protein and DNA [20,32]. The results of the ROS content of HT-29 cells in different groups are shown in Fig. 6. The green fluorescence points in the cells exposed to lead ion stimulation is obvious, indicating that the ROS level is significantly increased. After the intervention, treatment, and prevention with CT@RPS/CS, the green fluorescence point of cells in the visual field was not obvious, indicating that CT@RPS/CS can reduce ROS content in HT-29 cells. In addition, in the blank group and only the CT@RPS/CS group, the green fluorescence point of cells in the visual field is not apparent, indicating that the above treatment will not cause oxidative damage to HT-29 cells.

The results of SOD activity and MDA concentration of HT-29 cells in different groups are shown in Fig. 7. In the G-Pb group, the activity of SOD decreased significantly, and the content of MDA increased. This result is consistent with that reported in the literature [33,34]. In the G-C@R/C group, MDA did not increase or decrease significantly. However, in the intervention group, treatment group, and prevention group, SOD content increased, indicating that CT@RPS/CS can improve the activity of SOD enzyme without increasing MDA content, reflecting that CT@RPS/CS has a certain ability to antioxidant stress and can alleviate the oxidative stress caused by exposure to lead ion to a certain extent. Among them, in the intervention group, the values of SOD and MDA were similar to those in the G-Pb group, which reflected that the antioxidant stress protection ability of HT-29 cells was weak in the presence of Pb (II) and CT@RPS/CS at the same time. In the treatment group, the values of SOD and MDA were similar to those in the G-Pb group, reflecting that CT@RPS/CS could not repair the damaged cells of HT-29 cells exposed to Pb (II). In the prevention group, the SOD value and MDA value were like those in the Ctrl group, the SOD value was higher than that in the G-Pb group, and the MDA value was lower than that in the G-Pb group, which reflected that when CT@RPS/CS was added in advance, CT@RPS/CS and Pb (II) were preferentially combined. The combined compound could not effectively damage HT-29 cells and played a preventive and protective role.

Cytokines are substances synthesized under external stimulation and have physiological effects and immune regulation functions, including some low molecular weight proteins [35]. The changes in cytokine levels in intestinal epithelial cells are closely related to their immune status. Abnormal cytokine levels often reflect intestinal inflammatory response, increased permeability, and other physiological disorders [36–38]. In this study, the cytokines detected include pro-inflammatory factors and anti-inflammatory factors. The excessive production of inflammatory factors is one of the leading causes of tissue injury and TNF- α is one of the most critical inflammatory factors and has a wide range of biological effects. The expression level of pro-inflammatory cytokines decreased because IL-10 causes activated monocytes to produce IL-1 β, IL-6, IL-8, and TNF- α. In this study, the HT-29 cell model was used and exposed to

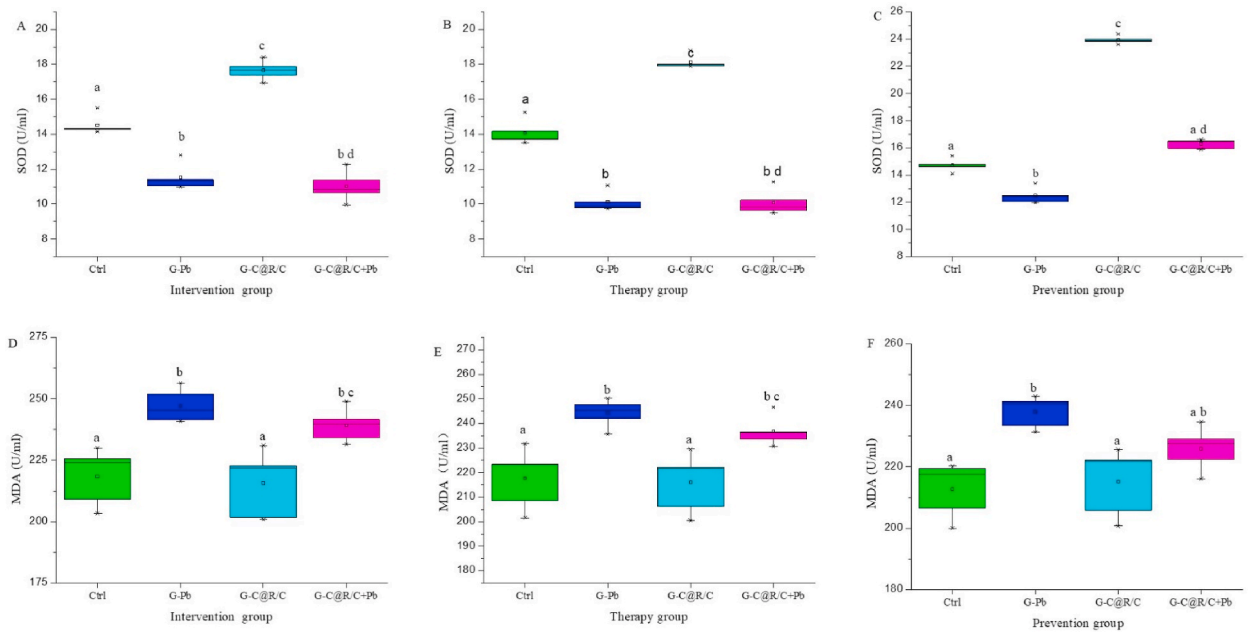

Fig. 7. SOD enzyme activity (A, B, C) and MDA expression (D, E, F) in the intervention group, treatment group, and prevention group. Note: a, b, c, and d represent statistical differences in intra-group comparisons, respectively.

Pb(II), and the expression of IL-1β, IL-6, IL-8, TNF-α, and IL-10 in Pb(II) exposed cells increased a lot compared with the Ctrl group, and there were significant differences in the comparison, suggesting that Pb(II) exposure may also be one of the factors causing the inflammatory response of intestinal cells. IL-10, as an inhibitory factor, increased the expression level under the condition of Pb (II) exposure in this study, which should have been able to reduce IL-1 β, IL-6, IL-8, and TNF-α. From the experimental results, the Pb (II) expression failed to show its due function. It may be that Pb (II) exposure led to the denaturation of intracellular proteins, disrupted the stability of the intracellular system, made intracellular cytokines unable to achieve dynamic balance, and caused intestinal cell inflammation. CT@RPS/CS was used to intervene, treat, and prevent Pb (II) exposed HT-29 cells. The results showed that the production of IL-1β, IL-6, IL-8, and TNF-α in the G-PB + C@R/C group decreased to varying degrees, reflecting that CT@RPS/CS can effectively restore the activity of HT-29 cells, stabilize the cell microenvironment, and play a better barrier role.

5. Conclusion

This study found that Pb (II) exposure can significantly reduce the activity of HT-29 cells, induce the level of antioxidant stress in HT-29 cells, and promote the body's absorption of lead. We found that CT@RPS/CS can effectively restore the activity of HT-29 cells after Pb (II) exposure and reduce ROS induced by Pb (II), reduce the production of relevant inflammatory cytokines, further play a certain protective role, and reduce the absorption of Pb (II) by cells, to reduce the toxicological role of Pb(II) in the host.

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Additional information

No additional information is available for this paper.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Suwei Jiang: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Hailiang Hu:** Writing – review & editing, Writing – original

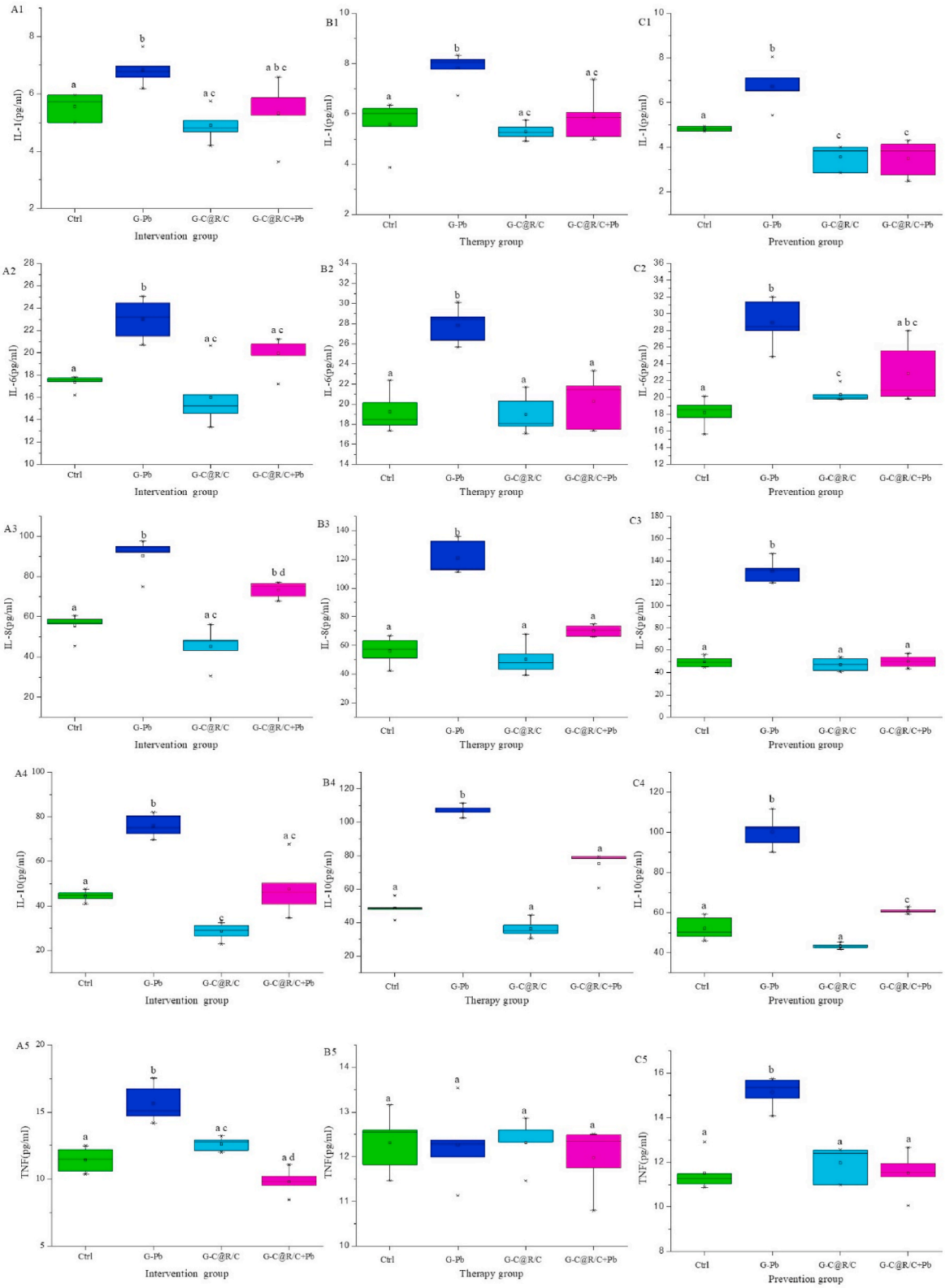

Fig. 8. Cytokines in the intervention group, therapy group, and prevention group. Note: a, b, c, and d represent statistical differences in intra-group comparisons, respectively A, B, and C represent intervention, therapy, and prevention groups. 1, 2, 3, 4 and 5 represent IL-1, IL-6, IL-8, IL-10, and TNF, respectively.

draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

References

[1] WHOTEAM, Chemical SafetyHealth Unit (CHE). Exposure to lead: a major public health concern, WHO REFERENCE NUMBER: WHO/CED/PHE/EPE/19.4.7. https://www.who.int/publications-detail-redirect/9789240037632.
[2] S. Yi, W. Jun, W. Yongning, Study on current situation and development trends of domestic and foreign lead maximum level standards in food, Journal of Food Safety and Quality (1) (2014) 294–299 (in chinese).
[3] W. Maoqi, et al., Study on food contaminants monitoring in China during 2000-2001, J. Hyg. Res. 32 (4) (2003) 322–326 (in chinese).
[4] A.L. Wani, A. Ara, J.A. Usmani, Lead toxicity: a review, Interdiscipl. Toxicol. 8 (2) (2015) 55–64.
[5] H. Ottenwälder, P. Simon, Differential effect of N-acetylcysteine on excretion of the metals Hg, Cd, Pb and Au, Arch. Toxicol. 60 (5) (1987) 401–402.
[6] W.H. Bett, Y. Zhang, Hj D, N-aeetyleysteine and captopril inhibit but Snitroso-N-acetyl cysteine stimulates human neutrophil free radical produetion, Cireulation (1993) 88–90.
[7] N. Ercal, et al., N-actylcysteine protects Chinese hamster ovary (CHO) cells from lead-induced oxidative stress, Toxicology 108 (1–2) (1996) 57–64.
[8] W.R. Yao, H.Y. Yao, Adsorbent characteristics of porous starch, Starch - Stärke 54 (6) (2002) 260–263.
[9] H.Q. Mao, et al., Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency, J. Contr. Release 70 (3) (2001) 399–421.
[10] X. Li, et al., Poly-DL-lactide–poly(ethylene glycol) microspheres as oral and parenteral delivery systems for hepatitis B surface antigen, J. Appl. Polym. Sci. 83 (4) (2010) 850–856.
[11] S. Avivi, et al., The preparation of magnetic proteinaceous microspheres using the sonochemical method, Biochim. Biophys. Acta Gen. Subj. 1527 (3) (2001) 123–129.
[12] A.I. Haza, P. Morales, Effects of (+)-catechin and (-)-epicatechin on heterocyclic amines-induced oxidative DNA damage, J. Appl. Toxicol. 31 (1) (2011) 53–62.
[13] W. YanJiang, et al., Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer's disease mouse, Neurotox. Res. 15 (1) (2009) 3–14.
[14] S. Mona, P.K. T, Fighting against the dark side of neutrophil extracellular traps in disease: manoeuvres for host protection, Curr. Opin. Hematol. 20 (1) (2013) 3–9.
[15] W. Feng, et al., Coordinated regulation of murine cardiomyocyte contractility by nanomolar (-)-epigallocatechin-3-gallate, the major green tea catechin, Mol. Pharmacol. 82 (5) (2012) 993–1000.
[16] Y.-J. Lee, et al., Epigallocatechin-3-gallate prevents systemic inflammation-induced memory deficiency and amyloidogenesis via its anti-neuroinflammatory properties, J. Nutr. Biochem. 24 (1) (2013) 298–310.
[17] D.E. Ehrnhoefer, et al., EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers[J], Nat. Struct. Mol. Biol. 15 (6) (2008) 558–566.
[18] M. Peng, S. Shi, Y. Zhang, Influence of Cd 2+ , Hg 2+ and Pb 2+ on (+)-catechin binding to bovine serum albumin studied by fluorescence spectroscopic methods, Spectrochim. Acta Part A Molecular & Biomolecular Spectroscopy 85 (1) (2012) 190–197.
[19] J.-H. Choi, et al., Action of green tea catechin on bone metabolic disorder in chronic cadmium-poisoned rats, Life Sci. 73 (12) (2003) 1479–1489.
[20] L. Chen, et al., Tea catechins protect against lead-induced cytotoxicity, lipid peroxidation, and membrane fluidity in HepG2 cells, Toxicol. Sci. 69 (1) (2002) 149–156.
[21] S. Jiang, H. Hu, Characteristics of catechin loading rice porous starch/chitosan functional microsphere and its adsorption towards Pb2+, Heliyon 8 (8) (2022) e10048.
[22] S. Khan, et al., Evaluation of in vitro cytotoxicity, biocompatibility, and changes in the expression of apoptosis regulatory proteins induced by cerium oxide nanocrystals, Sci. Technol. Adv. Mater. 18 (1) (2017) 364–373.
[23] S. Khan, et al., In vitro evaluation of anticancer and biological activities of synthesized manganese oxide nanoparticles, Medicinal Chemistry Communication 10 (2016) 1647–1653.
[24] S. Khan, et al., Design, synthesis and in vitro evaluation of anticancer and antibacterial potential of surface modified Tb(OH)3@SiO2 core–shell nanoparticles, RSC Adv. 6 (22) (2016) 18667–18677.
[25] S. Jiang, et al., Adsorption of procyanidins onto chitosan-modified porous rice starch, LWT 84 (2017) 10–17.
[26] L. Yu, et al., Dose-dependent effects of lead induced gut injuries: an in vitro and in vivo study, Chemosphere 266 (2021) 129130.
[27] J. Guo, et al., Anticancer effect of tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-O-1H-imidazole-3-cationic-1-oxy l-2)-pyrrolidine-1-carboxylic ester on human hepatoma HepG2 cell line, Chem. Biol. Interact. 199 (1) (2012) 38–48.
[28] V. Matovi, et al., Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys, Food Chem. Toxicol. 78 (2015) 130–140.
[29] F. Ye, et al., The role of Nrf2 in protection against Pb-induced oxidative stress and apoptosis in SH-SY5Y cells, Food Chem. Toxicol. 86 (2015) 191–201.
[30] B. Kumar, A. Kowluru, R.A. Kowluru, Lipotoxicity augments glucotoxicity-induced mitochondrial damage in the development of diabetic retinopathy, Invest. Ophthalmol. Vis. Sci. 56 (5) (2015) 2985–2992.
[31] Y. Shu, et al., Response of the common cutworm Spodoptera litura to lead stress: changes in sex ratio, Pb accumulations, midgut cell ultrastructure, Chemosphere 139 (2015) 441–451.
[32] G L, et al., Puerarin protects against lead-induced cytotoxicity in cultured primary rat proximal tubular cells, Hum. Exp. Toxicol. 33 (10) (2014) 1071–1080.
[33] C. Naifu, J. Hongwu, H. Xinhua, Effects of Pteridium aquilinum flavones on cell oxidize induced by lead stress in mice, Chinese Journal of Basic Medicine in Traditional Chinese Medicine 13 (2) (2007) 120–123 (in Chinese).
[34] L. Yun, et al., Effects of extremely low frequency electromagnefic field and its combination with lead on the antioxidant system in mouse, Chin. J. Ind. Hyg. Occup. Dis. 20 (4) (2002) 263–265 (in Chinese).
[35] H. Bruunsgaard, M. Pedersen, B. Pedersen, Aging and proinflammatory cytokines, Curr. Opin. Hematol. 8 (3) (2001) 131–136.
[36] L.V. Hooper, J.I. Gordon, Commensal host-bacterial relationships in the gut, Science 292 (5519) (2001) 1115–1118.
[37] C.s. Eun, et al., Lactobacillus casei prevents impaired barrier function in intestinal epithelial cells[J], APMIS 119 (1) (2015) 49–56.
[38] K. Mitsuyama, et al., IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease, Clin. Exp. Immunol. 96 (3) (2010) 432–436.