Icariin Alleviates Bisphenol A Induced Disruption of Intestinal Epithelial Barrier by Maintaining Redox Homeostasis In Vivo and In Vitro
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ABSTRACT: Bisphenol A (BPA), a globally prevalent environmental contaminant, has been shown to have the potential to disrupt intestinal barrier function. This study explored the mechanisms of BPA-induced intestinal barrier dysfunction. In addition, the protective effect of the natural product icariin (ICA) on BPA-induced intestinal barrier dysfunction was evaluated. BPA relieved oxidative stress (reactive oxygen species (ROS), reactive nitrogen species (RNS), malondialdehyde (MDA), and hydrogen peroxide (H2O2)), suppressed antioxidant enzyme (superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and total antioxidant capacity (T-AOC)) activity, and increased gene expression and protein content of p38 mitogen-activated protein kinase (MAPK), giving rise to the dysfunctional gut in mice. ICA therapy effectively eased intestinal barrier dysfunction caused by BPA in vivo and in vitro. Treatment with p38 MAPK inhibitor (SB203580) significantly rescued the MODE-K cell barrier function disrupted by BPA challenge. However, treatment with p38 MAPK activator (anisomycin) did not attenuate the MODE-K cell barrier function impaired by BPA challenge. Overall, our data suggested that BPA disrupted intestinal barrier function in a p38 MAPK-dependent manner. Furthermore, we demonstrated that ICA regulated the redox equilibrium of intestinal epithelial cells by inhibiting the expression of p38 MAPK, thereby alleviating BPA-induced disruption of intestinal barrier function. These findings contributed to a better understanding of the mechanisms of BPA-induced intestinal barrier dysfunction and provided new insights into the prevention and treatment of BPA-induced intestinal diseases.

INTRODUCTION
Bisphenol A (4,4′-(propane-2,2-diyl)diphenol; BPA), one of the most widely used industrial compounds in the world, is mainly used in the production of various polymers. BPA is applied to baby bottles, toys, sealant, eyewear lenses, and paper consumer products.1 It is the high production and consumption that makes it a widespread pollutant in the global environment.2−5 When plastic products are heated or exposed to ultraviolet (UV) light, BPA is released from the polymer into food and water,6 resulting in frequent exposure to BPA. Adverse effects of BPA on human health have attracted high attention in the field of public health because specific tissues and organs are highly susceptible to the toxic effects of BPA.7−9 A number of studies have found that BPA is widely found in food, environmental, and even biological samples.10−14 Toxic substances in intestinal lumen can induce dysfunction of intestinal epithelial cells, allowing harmful substances to escape and pass into the bloodstream, causing systemic metabolic diseases.15−17 Maintaining a good intestinal barrier function is critical to human health. The tight junction (TJ) complex is the main component of the intestinal epithelial barrier.18 Abnormal expression and distribution of tightly coupled complexes in intestinal epithelial cells will directly affect the intestinal barrier function and intestinal permeability, thus inducing many intestinal diseases.19 Researchers have done a lot of work on BPA and intestinal diseases and their relationship. The effects of BPA on gut health are mainly manifested in the downregulated lysozyme expression, decreased fecal antibacterial activity, reduced secretion level of intestine immunoglobulin A (IgA), increased intestinal permeability, and the occurrence of colitis.20,21 In addition, evidence shows that exposure to BPA destroyed the morphological structure of the intestinal epithelium, reduced the number of goblet cells, suppressed the expression of tight junction (TJ) protein, increased the permeability of the

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intestinal epithelium, and ultimately leading to impaired intestinal barrier function.

Previous studies have shown that oxidative stress is a critical biological process by which BPA mediates damage to gut barrier function. However, the mechanism by which BPA induces oxidative stress is unknown. A recent study revealed that p38 mitogen-activated protein kinase (MAPK) signaling pathway involved the regulation of oxidative stress and was closely related to the intestinal epithelial barrier function. Due to its safety and low cost, natural products have been

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**Figure 1.** Effects of ICA on jejunal permeability and barrier function in BPA-exposed mice. Plasma endotoxin (A), DAO (B), α-lactate (C), and zonulin (D) levels. Jejunal endotoxin (E), DAO (F), α-lactate (G), and zonulin (H) levels. Jejunal gene expressions of ZO-1 (I), occludin (J), and claudin-1 (K) detected by RT-qPCR. Values are the mean ± standard error (n = 10). *p < 0.05 vs CON group; †p < 0.05 vs BPA group.

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**Figure 2.** Effects of ICA on jejunal redox status in BPA-exposed mice. Plasma levels of ROS (A), RNS (B), MDA (C), and H_{2}O_{2} (D). Jejunal ROS (E), RNS (F), MDA (G), and H_{2}O_{2} (H) levels. Plasma SOD (I), GPx (J), CAT (K), and T-AOC (L) activity. Jejunal SOD (M), GPx (N), CAT (O), and T-AOC (P) activity. Values are the mean ± standard error (n = 10). *p < 0.05 vs CON group; †p < 0.05 vs BPA group.
widely used in disease resistance research. Icariin (ICA) is a representative natural product. ICA, a flavonoid extracted from epimedium, has a wide range of biological activities as well as pharmacological effects, mainly anti-inflammatory and antioxidant. Previous studies have shown that ICA can significantly alleviate intestinal injury induced by LPS, suggesting that ICA may have an outstanding protective effect on intestinal epithelium.26,27 Therefore, we hypothesized that ICA positively protects against BPA-induced intestinal epithelial barrier damage through regulation of the expression of p38 MAPK. To demonstrate this, we investigated the protective effect of ICA on intestinal epithelial barrier function by constructing in vivo and in vitro models induced by BPA. Moreover, the biological mechanism by which ICA has a protective effect on the intestinal epithelial barrier was elucidated through specific blockade or activation of the p38 MAPK signaling pathway.

### RESULTS

Effects of ICA on Jejunal Permeability and Barrier Function in BPA-Exposed Mice. To assess the effects of ICA on jejunal permeability and barrier function after BPA exposure, we examined the levels of the chemical markers (endotoxin, diamine peroxidase (DAO), d-lactate, and zonulin) and the gene expressions of tight junction proteins (ZO-1, occludin, and claudin-1). BPA exposure markedly increased the levels of these chemical markers in the plasma and jejunum, while ICA + BPA co-treatment decreased them compared with that in the BPA group ($p < 0.05$; Figure 1A–H). Besides, we used real-time quantitative polymerase chain reaction (RT-qPCR) to detect TJ-related gene expression and found that BPA exposure significantly reduced the gene expressions of ZO-1, occludin, and claudin-1 in the jejunal samples, while ICA + BPA co-treatment rescued the expressions of these tight junction proteins ($p < 0.05$; Figure 2A–G).
These results suggested that ICA can effectively alleviate BPA-induced intestinal barrier functional impairment.

**Effects of ICA on Jejunal Redox Status in BPA-Exposed Mice.** To investigate the effect of ICA on redox status in the jejunal epithelium after BPA exposure, we detected the levels of the chemical markers (reactive oxygen species (ROS), reactive nitrogen species (RNS), malondialdehyde (MDA), hydrogen peroxide (H$_2$O$_2$), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and total antioxidant capacity (T-AOC)) in plasma and jejunal samples of mice. BPA exposure significantly increased the levels of ROS, RNS, MDA, and H$_2$O$_2$ in plasma and jejunum, while ICA decreased the ROS, RNS, MDA, and H$_2$O$_2$ contents compared to the BPA group ($p < 0.05$; Figure 2A–H). Besides, BPA exposure markedly reduced the activity of SOD, GPx, CAT, and T-AOC in the plasma and jejunum, while ICA
+ BPA co-treatment increased the SOD, GPx, CAT, and T-AOC activity compared with those of the BPA group (p < 0.05; Figure 2I−P). These data demonstrated that ICA can reverse BPA-induced redox imbalances.

**Effects of ICA on Jejunal p38 MAPK Expression in BPA-Exposed Mice.** To reveal the influence of ICA on the expression of p38 MAPK in the jejunal epithelium after BPA exposure, we detected the mRNA expression and content of the p38 MAPK in jejunal samples of mice. As shown in Figure 3, mice challenged with BPA had a higher level of p38 MAPK gene expression and content than the CON group (p < 0.05; Figure 3A,B). Besides, the p38 MAPK gene expression and content in the ICA co-treatment group was significantly lower than that of the BPA group (p < 0.05; Figure 3A,B). These results suggested that p38 MAPK may be a key factor of ICA in alleviating BPA-induced intestinal injury.

**Effect of ICA on Redox Equilibrium in MODE-K Cells after BPA Challenge.** To evaluate the potential protective effect of ICA on redox balance with the BPA challenge, we also used 40 μg/mL ICA to process cells with 200 μM BPA for 24 h. To assess the redox state of MODE-K cells, mitochondrial and intracellular ROS levels, and a series of indicators (MDA, H2O2, SOD, GPx, CAT, and T-AOC) were measured. The level of mitochondrial and intracellular ROS, MDA, and H2O2 in MODE-K cells increased after the BPA challenge; meanwhile, the mitochondrial and intracellular ROS, MDA, and H2O2 contents in the BPA + ICA group was significantly lower than that in the BPA group (p < 0.05; Figure 5A−D). In addition, the SOD, GPx, CAT, and T-AOC activity in the ICA co-treatment group was markedly higher than that in the CON group; meanwhile, the LDH activity of the ICA + BPA co-treatment group was markedly lower than that of the BPA group (p < 0.05; Figure 4B). In addition, the BPA treatment reduced the value of trans-
epithelial electrical resistance (TEER) and increased the fluorescein isothiocyanate-dextran (FITC-D4) flux of MODE-K cell (p < 0.05; Figure 4C,D). Compared to the BPA group, the value of TEER was relatively elevated after the ICA co-treatment, while the FITC-D4 flux was relatively declined (p < 0.05; Figure 4C,D). Besides, we used RT-qPCR to detect TJ-related gene expression and found that the challenge with BPA decreased ZO-1, occludin, and claudin-1 gene expression in MODE-K cells, while the gene expressions of ZO-1, occludin, and claudin-1 in the BPA + ICA group were significantly higher than those in the BPA group (p < 0.05; Figure 4E−G). These results validated the efficacy of ICA against the intestinal toxicity of BPA in vitro.

**Figure 7. Effect of p38 MAPK inhibitor on cell viability, barrier function, and oxidative stress in MODE-K cells with BPA challenge. (A) Cells viability and (B) LDH activity of MODE-K cells. (C) TEER and (D) FITC-D4 of MODE-K cells. Changes in the levels of (E) mitochondrial ROS (MitoSOX dye oxidation) and (F) total intracellular ROS (H2DCF oxidation) in MODE-K cells. mRNA expression of (G) ZO-1, (H) occludin, and (I) claudin-1 in MODE-K cells detected by RT-qPCR. Values are the mean ± standard error (n = 6). *p < 0.05 vs CON group; †p < 0.05 vs BPA group.
These data validated the efficacy of ICA against BPA-induced redox disturbance in vitro.

**Effect of ICA on the Expression of p38 MAPK and Oxidative Stress in MODE-K Cells after BPA Challenge.**

As shown in Figure 6, MODE-K cells treated with BPA had a higher level of p38 MAPK gene expression and content than the CON group ($p < 0.05$; Figure 6A,B). Besides, the p38 MAPK gene expression and content of MODE-K cells in the ICA co-treatment group were significantly lower than those in the BPA group ($p < 0.05$; Figure 6A,B). To explore the underlying mechanisms by which ICA alleviates the disruption of the monolayer function of the MODE-K cells induced by BPA, we explored the p38 MAPK expression as well as oxidative status. Compared with the CON group, the ICA group significantly reduced the gene expression and content of p38 MAPK and decreased the mitochondrial and intracellular ROS levels in MODE-K cells ($p < 0.05$; Figure 6C–F). These data suggested that ICA may play a role in protecting the gut by regulating p38 MAPK in vitro.

**Effects of Co-treatment with ICA and p38 MAPK Inhibitor/Activator on Cell Viability, Barrier Function, and Oxidative Stress of MODE-K Cells after BPA Challenge.**

We employed inhibitors/activators of p38 MAPK to elucidate the potential molecular mechanisms by which ICA alleviates BPA-induced intestinal injury. As shown in Figure 7, when compared to the BPA group, the p38 MAPK inhibitor (SB203580) group significantly increased the MODE-K cells viability and LDH activity, increased the value of TEER, decreased the FITC-D4 flux, and reduced the levels of mitochondrial and intracellular ROS ($p < 0.05$; Figure 7A–F). Besides, compared with the BPA group, the p38 MAPK inhibitor (SB203580) group significantly increased the mRNA expression of ZO-1, occludin, and claudin-1 ($p < 0.05$; Figure 7G–I). As shown in Figure 8, compared with the CON group, the BPA group and the BPA + ICA + p38 MAPK activator (anisomycin) group significantly reduced the MODE-K cell viability and LDH activity, declined the value of TEER, increased the FITC-D4 flux, increased the mitochondrial and level of intracellular ROS, and reduced the mRNA expression of ZO-1, occludin, and claudin-1 ($p < 0.05$; Figure 8A–I). While compared to the BPA group, the BPA + ICA + p38 MAPK activator (anisomycin) group hardly had change in cell viability, barrier function, and oxidative stress. These results confirm that p38 MAPK is essential for ICA to play a role in combating BPA.

**DISCUSSION**

BPA, a substance that pollutes the environment in daily life, has been shown to cause potential damage to numerous human tissues and organs (lungs, liver, kidneys, skin, and mucous membranes) in the human body. A recent study has shown that mice exposed to BPA suffer from a severe intestinal disease, characterized by damage to the intestinal epithelium and breakdown of the intestinal barrier. Numerous studies have shown that ICA has an excellent preventive effect against a variety of diseases, which is mainly attributed to its excellent antioxidant properties. Therefore, we tried to figure out the positive effects of ICA on intestinal barrier function and its potential mechanisms of BPA challenge in this study.

The previous study has shown that BPA exposure increased intestinal epithelial histopathological score in mice and decreased the gene expression of tight junction proteins in the intestinal epithelium, thereby disrupting the intestinal barrier function. In addition, in vitro studies have shown that BPA-promoted apoptosis and inhibited proliferation of gut epithelial cells. The present data showed that BPA exposure significantly reduced the gene expression of tight junction proteins.
proteins (ZO-1, occludin, and claudin-1) in the jejunum of mice and MODE-K cells. In a tight junction complex, ZO-1 is a peripheral membrane protein, which plays an important role in the distribution and maintenance of tight junctions. Ocludin interacts directly with claudins and actin to promote the transfer of macromolecules through the cell bypass pathway. In addition, members of the claudins family also contribute to the functioning of the intestinal barrier. Therefore, ZO-1, occludin, and claudins play a crucial role in maintaining the intestinal barrier. Damage to the intestinal barrier caused by BPA increased the permeability of detrimental substances in the intestinal lumen.22 Our results showed a significant increase in plasma and jejunal endotoxin, DAO, β-lactic acid, and zonulin levels after BPA treatment. Generally speaking, for healthy individuals, indicators (endotoxin, DAO, β-lactic acid, and zonulin) are low in the circulatory system, which are significantly increased during the destruction of the intestinal wall.39 Besides, our data revealed that the BPA challenge decreased the transmembrane tolerance of MODE-K cells and increased the FITC-D4 flux. More importantly, treatment with ICA significantly reduced the damage to the intestinal barrier and permeability of mice and MODE-K cells induced by BPA. All these data suggested that BPA gives rise to increased intestinal permeability and damage to the intestinal barrier and permeability of mice and MODE-K cells induced by BPA. These effects indicated that BPA leads to impairment of intestinal epithelial barrier function in a p38 MAPK-dependent manner, and ICA rescues the intestinal epithelial barrier function by inhibiting BPA-induced p38 MAPK expression.

CONCLUSIONS

Our results demonstrated that the disorder of redox equilibrium induced by p38 MAPK activation is an essential step of BPA-induced intestinal epithelial barrier and permeability disruption. What is more important is that our results emphasize that ICA has a protective effect on the intestinal epithelial barrier dysfunction induced by BPA through regulating p38 MAPK expression. These data suggested that p38 MAPK is a key target for the prevention and treatment of BPA-induced intestinal diseases, and ICA may be an effective natural product for the prevention of intestinal damage caused by BPA.

MATERIALS AND METHODS

Reagents. BPA was purchased from Sigma (lot no. 239658). ICA (lot no. 20171125, net content 90.00%) was purchased from Xi’an Grassroot Chemical Engineering Co. Ltd. (Xian, China). SB203580 (lot no. GC13595, purity = 98.00%) and anisomycin (lot no. SC0132, purity = 99.00%) were obtained from GlpBio Technology and Beyotime Biotechnology (China), respectively.

Animal Maintenance and Experimental Designs. For this study, 40 male C57BL/6 mice aged 3 weeks were selected. All mice are kept in an environment free of specific pathogens. The experimental environment guarantees constant temperature and humidity, light/dark cycle for 12 h. Four groups of mice (n = 10 each) were used in this study. Group 1 (CON group) received only vehicle (filtered water). Group 2 (BPA group) were given BPA orally at 50 μg/kg day). Group 3 (BPA + ICA group) received both ICA (20 mg/kg day) and BPA (50 μg/kg day). All treatments were given daily for 10 weeks. To reduce sample variability, the intestinal segments were collected from the approximate middle position of the intestinal tract (jejunum). The jejunal epithelium was separated from the muscular layers by blunt dissection and stored at −80 °C prior to further analysis. Blood samples from mice were collected through the jugular vein and then collected into heparin anticoagulation tubes (5 mL). Plasma samples were then stored by centrifugation for 10 min (3000g, 4 °C) at −80 °C until further analysis.

Cell Culture. MODE-K cells are an intestinal epithelial cell line derived from C3H/HeJ mice, bought from Shanghai Cell Bank, Chinese Academy of Sciences (lot no. BFN608006456). MODE-K cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum, 1% penicillin/streptomycin, 1% gentamicin, 1% 4-(2-hydroxyethyl-1H-1,2,3-triazol-1-yl)ethanol (β-mercaptoethanol), and 1% non-essential amino acids. This study used the 20% order of the mixed of 10 μg/ml SB203580 and 20 μg/ml anisomycin. For this study, 40 male C57BL/6 mice aged 3 weeks were selected. All mice are kept in an environment free of specific pathogens. The experimental environment guarantees constant temperature and humidity, light/dark cycle for 12 h. Four groups of mice (n = 10 each) were used in this study. Group 1 (CON group) received only vehicle (filtered water). Group 2 (BPA group) were given BPA orally at 50 μg/kg day). Group 3 (BPA + ICA group) received both ICA (20 mg/kg day) and BPA (50 μg/kg day). All treatments were given daily for 10 weeks. To reduce sample variability, the intestinal segments were collected from the approximate middle position of the intestinal tract (jejunum). The jejunal epithelium was separated from the muscular layers by blunt dissection and stored at −80 °C prior to further analysis.
yl)-1-piperazineethanesulfonic acid (HEPES) buffer, and 1% nonessential amino acids. The medium was changed every 2–3 days. The incubation conditions were 37 °C and a 5% CO₂ atmosphere.

**Determination of Jejunal Permeability.** The endotoxin, diamine peroxidase (DAO), d-lactate, and zonulin content in plasma and jejunal samples were determined using commercial kits (Shanghai Enzyme-Linked Biotechnology Co. Ltd., Shanghai, China). The detailed steps of the test operation are given in the manufacturer’s instructions.

**Determination of Jejunal Oxidative Status.** Reactive oxygen species (ROS), reactive nitrogen species (RNS), malondialdehyde (MDA), and hydrogen peroxide (H₂O₂) contents in plasma and jejunal samples were determined using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-Linked Biotechnology Co. Ltd., Shanghai, China). The detailed steps of the test operation are given in the manufacturer’s instructions.

**Determination of MODE-K Cells’ Oxidative Status.** Intracellular reactive oxygen species (ROS) (in the MODE-K cells were measured using 2',7'-dichlorofluorescein diacetate (DCFH-DA; Sigma) per a previously reported method. In brief, the cells were washed three times with phosphate-buffered saline (PBS) after removing the culture medium. A 10 μM DCFH-DA solution was added to the cells and incubated for 30 min at 37 °C. Next, the cells were washed three times with PBS. Then, the cells were resuspended in 1 mL of PBS and total intracellular fluorescence intensity was measured by flow cytometry (FACS Verse, BD Biosciences, San Jose, CA). The level of total intracellular ROS paralleled the increase in fluorescence intensity and was calculated as the percentage of control cells.

Mitochondrial ROS in the MODE-K cells were measured using MitoSOX Red mitochondrial superoxide indicator (Invitrogen) as described previously. Briefly, the cells were washed three times with PBS after the culture medium. MitoSOX Red mitochondrial superoxide indicator, diluted to a final concentration of 4 mM in serum-free DMEM, was added to the cells and incubated for 30 min at 37 °C. The cells were washed three times with PBS. Then, the cells were resuspended in PBS, and fluorescence was measured immediately with a flow cytometer. The level of mitochondrial ROS paralleled the increase in fluorescence and was calculated as the percentage of control cells.

**Determination of Jejunal and MODE-K Cells’ Anti-oxidative Status.** The superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) activity, and total antioxidant capacity (T-AOC) in plasma, jejunal, and MODE-K cell samples were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China). The detailed steps of the test operation are given in the manufacturer’s instructions.

**Determination of Jejunal and MODE-K Cells’ p38 MAPK Content.** The p38 MAPK contents in jejunal and MODE-K cell samples were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China). The detailed steps of the test operation are given in the manufacturer’s instructions.

**Cell Viability Assay.** Cell viability was tested as described previously. Briefly, the cells were cultured for 24 h in 96-well plates. After treatment, 10 μL of the CCK-8 assay solution was added to each well and incubated for another 1 h. Then, the optical densities were read on a microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm. Lactate dehydrogenase (LDH) measurements were also used to assess cell viability. The cells were cultured for 24 h in 96-well plates. After treatment, the LDH content was determined using an assay kit. Then, the optical densities were read on a microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm. Cell viability is presented relative to the control group.

**MODE-K Cell Monolayer’s Barrier Function.** The MODE-K cell monolayer was constructed by seeding 0.2 × 10⁶ cells into each well of a 24-well Transwell plate (Corning, Inc., Corning, NY). The insertion area was 0.33 cm², and the pore size was 0.4 μm. The culture medium was changed every other day. Cells reached confluence on day 2, and the treatments were performed on day 7. The transepithelial electrical resistance (TEER) value of the MODE-K monolayers reached approximately 150 Ω·cm² at 7 days after confluence. Fluorescein isothiocyanate-dextran (FITC-D4, 4 kDa, 0.25 mM) measurements were taken for paracellular permeability. FITC-D4 was added to the apical chamber at the end of the treatment. After 2 h, 50 μL of the medium from the bottom chamber was transferred to a fluorescence measurement plate, and the fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. TEER and FITC-D4 flux values are both expressed as percentages of the control cells.

**RNA Isolation, cDNA Synthesis, and Real-Time Quantitative PCR.** Total RNA was extracted from each jejunal tissue or cell sample using TRIzol reagent. The RNA concentration and quality in the extracted colonic samples were measured using a NanoDrop ND-1000 spectrophotometer (Thermo). Next, 2 μg of total RNA was treated with RNase-Free DNase and reverse transcribed per the manufacturer’s instructions. Diluted cDNA (2 μL; 1:20, v/v) was used for real-time PCR, which was performed using an Mx3000P real-time PCR system (Stratagene). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was unaffected by the experimental factors, was chosen as the housekeeping gene. All primers used in this study are listed in Table 1.

**Table 1. Primer Sequences Used in This Study**

| target genes | primer forward/reverse | primer sequence (5’ → 3’) |
|--------------|------------------------|-------------------------|
| GAPDH        | forward/reverse         | TGCACCAACCAACTGCTTATGC/GGCATGGACTGTGGTCATGAG |
| ZO-1         | forward/reverse         | GCTCCTGCTATCCACCTATA/GCTAATCGGGGCTCTCATA |
| occludin     | forward/reverse         | GCCATTTGTAAGGACGAG/GACGTAAGCATTTGGCGCA |
| claudin-1    | forward/reverse         | GTGACCGCTGGACGGAAA/CTCCGGTGATTCACAGATTTC |
| caspase-3    | forward/reverse         | TGGAAATGTAGCGTGATGTT/GGCGGGCTGAATATGAAA |

were synthesized by Generay Company (Shanghai, China). The 2−ΔΔCt method was used to analyze the real-time PCR results, and gene mRNA levels are expressed as the fold change relative to the mean value of the control group.

**Statistical Analysis.** All data are presented as mean ± standard error of mean (SEM). Statistical significance was calculated by independent-sample t-test using SPSS (SPSS v. 20.0, SPSS Inc., Chicago, IL) software and accepted for p < 0.05. The numbers of the replicates are noted in the figures.
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Author Contributions
K.Z. performed the experiment and drafted the manuscript. Y.Z. analyzed the data. Y.Y. and Y.B. contributed to the experimental design and manuscript revision. T.Z. conceived the idea, designed the experiment, and finalized the manuscript. All authors read and approved the final manuscript.

Notes
The authors disclaim any competing financial interest.

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REFERENCES

(1) Mikołajewska, K.; Stragierowicz, J.; Gromadzińska, J. Bisphenol A - Application, sources of exposure and potential risks in infants, children and pregnant women. Int. J. Occup. Med. Environ. Health 2015, 28, 209–241.
(2) Huang, R. P.; Liu, Z. H.; Yuan, S. F.; Yin, H.; Dang, Z.; Wu, P. X. Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000–2016) and its risk analysis. Environ. Pollut. 2017, 230, 143–152.
(3) Di Bella, G.; Ben Mansour, H.; Ben Tekaya, A.; Belfaïa, A.; Potorti, A. G.; Saiya, E.; Bartolomeo, G.; Dugo, G.; Lo Turco, V. Plasticizers and BPA Residues in Tunisian and Italian Culinary Herbs and Spices. J. Food Sci. 2018, 83, 1769–1774.
(4) Lo Turco, V.; Potorti, A. G.; Ben Mansour, H.; Dugo, G.; Di Bella, G. Plasticizers and BPA in spices and aromatic herbs of Mediterranean areas. Nat. Prod. Res. 2020, 34, 87–92.
(5) Lo Turco, V.; Di Bella, G.; Potorti, A. G.; Tropea, A.; Casale, E. K.; Fede, M. R.; Dugo, G. Determination of plasticisers and BPA in Sicilian and Calabrian nectar honeys by selected ion monitoring GC/MS. Food Addit. Contam., Part A 2016, 33, 1693–1699.
(6) Le, H. H.; Carlson, E. M.; Chua, J. P.; Belcher, S. M. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. Toxicol. Lett. 2008, 176, 149–156.
(7) Rochester, J. R. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 2013, 42, 132–155.
(8) Fenichel, P.; Chevalier, N.; Brucker-Davis, F. Bisphenol A: an endocrine and metabolic disruptor. Ann. Endocrinol. 2013, 74, 211–220.
(9) Jang, J. W.; Lee, J. W.; Yoon, Y. D.; Kang, J. S.; Moon, E. Y. Bisphenol A and its substitutes regulate human B cell survival via Nrf2 expression. Environ. Pollut. 2020, 259, No. 113907.
(10) Cao, X. L.; Perez-Locas, C.; Dufresne, G.; Clement, G.; Popovic, S.; Beraldin, F.; Dabeka, R. W.; Feeley, M. Concentrations of bisphenol A in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates. Food Addit. Contam., Part A 2011, 28, 791–798.
(11) Valentino, R.; D’Esposito, V.; Ariemma, F.; Cimmino, L.; Beguinot, F.; Formisano, P. Bisphenol A environmental exposure and the detrimental effects on human metabolic health: is it necessary to revise the risk assessment in vulnerable population? J. Endocrinol. Invest. 2016, 39, 259–263.
(12) Fénichel, P.; Dechaux, H.; Harthe, C.; Gal, J.; Ferrari, P.; Pacini, P.; Wagner-Mahler, K.; Pugeat, M.; Brucker-Davis, F. Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes. Hum. Reprod. 2012, 27, 983–990.
(13) Mouneimne, Y.; Nasrallah, M.; Khoueiry-Zgheib, N.; Nasreddine, L.; Nakhou, N.; Ismail, H.; Abiad, M.; Kolelat, L.; Tamim, H. Bisphenol A urinary level, its correlates, and association with cardiometabolic risks in Lebanese urban adults. Environ. Monit. Assess. 2017, 189, No. 517.
(14) Zhou, Y.; Yao, Y.; Shao, Y.; Qu, W.; Chen, Y.; Jiang, Q. Urinary bisphenol analogues concentrations and biomarkers of oxidative DNA and RNA damage in Chinese school children in East China: A repeated measures study. Environ. Pollut. 2019, 254, No. 112921.
(15) Salvo Romero, E.; Alonso Cotoner, C.; Pardo Camacho, C.; Casado Bedmar, M.; Vicario, M. The intestinal barrier function and its involvement in digestive disease. Rev. Esp. Enferm. Dig. 2015, 107, 866–896.
(16) Bischoff, S. C.; Barbara, G.; Buurman, W.; Ochkuizen, T.; Schulzke, J. D.; Serino, M.; Tüllg, H.; Watson, A.; Wells, J. M. Intestinal permeability—a new target for disease prevention and therapy. BMC Gastroenterol. 2014, 14, No. 189.
(17) Tinkov, A. A.; Gritsenko, V. A.; Skalnaya, M. G.; Cherkasov, S. V.; Aaseth, J.; Skalny, A. V. Gut as a target for cadmium toxicity. Environ. Pollut. 2018, 235, 429–434.
(18) González-Castro, A. M.; Martínez, C.; Salvo-Romero, E.; Forteza, M.; Pardo-Camacho, C.; Perez-Berezo, T.; Alonso-Cotoner, C.; Santos, J.; Vicario, M. Mucosal pathobiology and molecular signature of epithelial barrier dysfunction in the small intestine in irritable bowel syndrome. J. Gastroenterol. Hepatol. 2017, 32, 53–63.
(19) Tao, S.; Bai, Y.; Li, T.; Li, N.; Wang, J. Original low birth weight deteriorates the hindgut epithelial barrier function in pigs at the growing stage. Front. Microbiol. 2019, 33, 9907–9912.
(20) Braniste, V.; Jouault, A.; Gaultier, E.; Polizzi, A.; Buisson-Brenac, C.; Leveque, M.; Martin, P. G.; Theodorou, V.; Fioramonti, J.; Houdeau, E. Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 448–453.
(21) Malaisé, Y.; Menard, S.; Cartier, C.; Lencina, C.; Sommer, C.; Gaultier, E.; Houdeau, E.; Guzy-Jack-Piriou, L. Consequences of bisphenol a perinatal exposure on immune responses and gut barrier function in mice. Arch. Toxicol. 2018, 92, 347–358.
(22) Feng, L.; Chen, S.; Zhang, L.; Qu, W.; Chen, Z. Bisphenol A increases intestinal permeability through disrupting intestinal barrier function in mice. Environ. Pollut. 2019, 254, No. 112960.
(23) Wang, K.; Zhao, Z.; Ji, W. Bisphenol A induces apoptosis, oxidative stress and inflammatory response in colon and liver of mice in a mitochondria-dependent manner. Biomed. Pharmacother. 2019, 117, No. 109182.
(24) Uwada, J.; Yazawa, T.; Islam, M. T.; Khan, M. R. I.; Krug, S. M.; Fromm, M.; Karaki, S. I.; Suzuki, Y.; Kuwahara, A.; Yoshide, H.; Sada, K.; Muramatsu, I.; Taniguchi, T.; Y.; Wang, Z. Icariin and its phosphorylated derivatives alleviate oxidative stress and inflammatory response in colon and liver of mice in a mitochondria-dependent manner. Biomed. Pharmacother. 2019, 117, No. 109182.
intestinal epithelial barrier disruption caused by enterotoxigenic *Escherichia coli* through modulate p38 MAPK in vivo and in vitro. *FASEB J.* 2020, 34, 1783–1801.

27. Xiong, W.; Ma, H.; Zhang, Z.; Jin, M.; Wang, J.; Xu, Y.; Wang, Z. The protective effect of icarin and phosphorylated icarin against LPS-induced intestinal epithelial cells injury. *Biomed. Pharmacother.* 2019, 118, No. 109246.

(28) Soundararajan, A.; Prabu, P.; Mohan, V.; Gibert, Y.; Balasubramanym, M. Novel insights of elevated systemic levels of bisphenol-A (BPA) linked to poor glycemic control, accelerated metabolic and histopathological changes in male Wistar albino rats: protective role of boron. *Toxicon.* Res. 2019, 8, 262–269.

30. Beltifa, A.; Feriani, A.; Machrekhi, M.; Ghorbel, A.; Ghazouani, L.; Di Bella, G.; Van Loco, J.; Reyns, T.; Mansour, H. B. Plasticizers and bisphenol A in packaged foods sold in the Tunisian markets: study of their acute in vivo toxicity and their environmental fate. *Environ. Sci. Pollut. Res.* 2017, 24, 22382–22392.

31. Beltifa, A.; Feriani, A.; Machrekhi, M.; Ghorbel, A.; Ghazouani, L.; Di Bella, G.; Sire, O.; Van Loco, J.; Reyns, T.; Mansour, H. B. Persistent plasticizers and bisphenol in the cheese of Tunisian markets induced biochemical and histopathological alterations in male BALB/c mice. *Environ. Sci. Pollut. Res.* 2018, 25, 6545–6557.

32. Hua, W.; Zhang, Y.; Wu, X.; Kang, L.; Tu, J.; Zhao, K.; Li, S.; Wang, K.; Song, Y.; Luo, R.; Shao, Z.; Yang, S.; Yang, C. Icarin Attenuates Interleukin-1beta-Induced Inflammatory Response in Human Nucleus Pulposus Cells. *Curr. Pharm. Des.* 2018, 23, 6071–6078.

33. Song, Y. H.; Cai, H.; Zhao, Z. M.; Chang, W. J.; Gu, N.; Cao, S. P.; Wu, M. L. Icarin attenuated oxidative stress induced-cardiac apoptosis by mitochondria protection and ERK activation. *Biomed. Pharmacother.* 2016, 83, 1089–1094.

34. Qu, W.; Zhao, Z.; Chen, S.; Zheng, L.; Wu, D.; Chen, Z. Bisphenol A suppresses proliferation and induces apoptosis in colonic epithelial cells through mitochondrial and MAPK/ARK pathways. *Life Sci.* 2018, 208, 167–174.

35. Palatinus, J. A.; O’Quinn, M. P.; Barker, R. J.; Harris, B. S.; Jordan, J.; Gourdie, R. G. ZO-1 determines adherens and gap junction localization at intercalated disks. *Am. J. Physiol.: Heart Circ. Physiol.* 2011, 300, H583–H594.

36. Al-Sadi, R.; Khatib, K.; Guo, S.; Ye, D.; Youssef, M.; Ma, T. Occludin regulates macromolecule flux across the intestinal epithelial tight junction barrier. *Am. J. Physiol.: Gastrointest. Liver Physiol.* 2011, 300, G1054–G1064.

37. Wu, Y.; Zhu, C.; Chen, Z.; Chen, Z.; Zhang, W.; Ma, X.; Wang, L.; Yang, X.; Jiang, Z. Protective effects of *Lactobacillus plantarum* on epithelial barrier disruption caused by enterotoxigenic *Escherichia coli* in intestinal porcine epithelial cells. *Vet. Immunol. Immunopathol.* 2016, 172, 55–63.

38. Wang, H. B.; Wang, P. Y.; Wang, X.; Wan, Y. L.; Liu, Y. C. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig. Dis. Sci.* 2012, 57, 3126–3135.

39. Ji, J.; Gu, Z.; Li, H.; Su, L.; Liu, Z. Cryptdin-2 predicts intestinal injury during heatstroke in mice. *Int. J. Mol. Med.* 2018, 41, 137–146.

40. Minamiyama, Y.; Ichikawa, H.; Takemura, S.; Kusunoki, H.; Naito, Y.; Yoshikawa, T. Generation of reactive oxygen species in sperms of rats as an earlier marker for evaluating the toxicity of endocrine-disrupting chemicals. *Free Radical Res.* 2010, 44, 1398–1406.

41. Zeng, Q.; Yi, H.; Huang, L.; An, Q.; Wang, H. Long-term arsenite exposure induces testicular toxicity by redox imbalance, G2/M cell arrest and apoptosis in mice. *Toxicology* 2019, 411, 122–132.

42. Gangwar, R.; Meena, A. S.; Shukla, P. K.; Nagaraja, A. S.; Dorniak, P. L.; Pallikuth, S.; Waters, C. M.; Sood, A.; Rao, R. Calcium-mediated oxidative stress: a common mechanism in tight junction disruption by different types of cellular stress. *Biochem.* 2017, 474, 731–749.

43. Song, D.; Cheng, Y.; Li, X.; Wang, F.; Lu, Z.; Xiao, X.; Wang, Y. Biogenic Nanoparticles Effectively Attenuate Oxidative Stress-Induced Intestinal Epithelial Barrier Injury by Activating the Nrf2 Antioxidant Pathway. *ACS Appl. Mater. Interfaces* 2017, 9, 14724–14740.

44. Yu, H.; Ding, X.; Shang, L.; Zeng, X.; Liu, H.; Li, N.; Huang, S.; Wang, Y.; Wang, G.; Cai, S.; Chen, M.; Levesque, C. L.; Johnston, L. J.; Qiao, S. Protective Ability of Biogenic Antimicrobial Peptide Microcin J25 Against Enterotoxigenic *Escherichia coli*-Induced Intestinal Epithelial Dysfunction and Inflammatory Responses IPEC-J2 Cells. *Front. Cell. Infect. Microbiol.* 2018, 8, No. 242.

45. Wang, S.; Gu, J.; Xu, Z.; Zhang, Z.; Bai, T.; Xu, J.; Cai, J.; Barnes, G.; Liu, Q.; Freedman, J. H.; Wang, Y.; Liu, Q.; Zheng, Y.; Cai, L. Zinc rescues obesity-induced cardiac hypertrophy via stimulating metallothionein to suppress oxidative stress-activated BCL10/CARD9/p38 MAPK pathway. *J. Cell. Mol. Med.* 2017, 21, 1182–1192.

46. Tao, S.; Sun, Q.; Cai, L.; Geng, Y.; Hua, C.; Ni, Y.; Zhao, R. Caspase-1-dependent mechanism mediating the harmful impacts of the quorum-sensing molecule N-(3-oxo-dodecanoyl)-l-homoserine lactone on the intestinal cells. *J. Cell. Physiol.* 2019, 234, 3621–3633.

47. Tao, S.; Luo, Y.; Bin, H.; Liu, J.; Qian, X.; Ni, Y.; Zhao, R. Paraoxonase 2 modulates a proapoptotic function in LS174T cells in response to quorum sensing molecule N-(3-oxododecanoyl)-l-homoserine lactone. *Sci. Rep.* 2016, 6, No. 28778.

48. Wang, Y. Y.; Sun, S. P.; Zhu, H. S.; Jiao, X. Q.; Zhong, K.; Guo, Y. J.; Zha, G. M.; Han, L. Q.; Yang, G. Y.; Li, H. P. GABA regulates the proliferation and apoptosis of MAC-T cells through the LPS-induced TLR4 signaling pathway. *Res. Vet. Sci.* 2018, 118, 395–402.

49. Chen, T.; Kim, C. Y.; Kaur, A.; Lamothe, L.; Shaikh, M.; Keshavarzian, A.; Hamaker, B. R. Dietary fibre-based SCFA mixtures promote both protection and repair of intestinal epithelial barrier function in a Caco-2 cell model. *Food Funct.* 2017, 8, 1166–1173.