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Microplastics caused embryonic growth retardation and placental dysfunction in pregnant mice by activating GRP78/IRE1α/JNK axis induced apoptosis and endoplasmic reticulum stress

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Abstract

Microplastics (MPs), a brand-new class of worldwide environmental pollutant, have received a lot of attention. MPs are consumed by both humans and animals through water, food chain and other ways, which may cause potential health risks. However, the effects of MPs on embryonic development, especially placental function, and its related mechanisms still need to be further studied. We investigated the impact on fetal development and placental physiological function of pregnant mice by consecutive gavages of MPs at 0, 25, 50, 100 mg/kg body weight during gestational days (GDs 0–14). The results showed that continuous exposure to high concentrations of MP significantly reduced daily weight gain and impaired reproductive performance of pregnant mice. In addition, MPs could significantly induce oxidative stress and placental dysfunction in pregnant mice. On the other hand, MPs exposure significantly decreased placental barrier function and induced placental inflammation. Specifically, MPs treatment significantly reduced the expression of tight junction proteins in placentas, accompanied by inflammatory cell infiltration and increased mRNA levels of pro-inflammatory cytokines and chemokines in placentas. Finally, we found that MPs induced placental apoptosis and endoplasmic reticulum (ER) stress through the GRP78/IRE1α/JNK axis, leading to placental dysfunction and decreased reproductive performance in pregnant mice. We revealed for the first time that the effects of MPs on placental dysfunction in pregnant animals. Blocking the targets of MPs mediated ER stress will provide potential therapeutic ideas for the toxic effects of MPs on maternal pregnancy.

Keywords Microplastics, Pregnant mice, Placenta, Developmental toxicity, Endoplasmic reticulum stress, MAPK pathway

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Introduction

Plastic products are widely used all over the world because of their good properties such as durability and low price. Despite the massive amounts of plastic consumed, only 20% of plastic garbage generated globally is recycled or burned; the majority of plastic waste is dumped into landfills or the environment [1–3]. Due to the difficulty of plastic degradation, it lasts for decades or even hundreds of years in the environment, and is further broken into finer plastic fragments through physical wear, chemical reactions and biodegradation [4]. Thus, microplastics (MPs) emerged as a new type of pollutant, and MPs were subsequently defined as plastic pieces or particles with a diameter of less than 5 mm [5]. Studies have shown that MPs have already spread throughout the global ecosystem [6]. Besides, MPs can be also exposed to various organisms through breathing, eating, drinking and other ways, and continue to be transmitted through the food chain, eventually threatening human health [5, 7]. It is estimated that the total amount of MPs ingested by each person through the digestive and respiratory pathways can range from 74,000 to 121,000 per year [8]. Additionally, research has revealed that MPs can accumulate in multiple organs such as liver, spleen, kidney and intestine, which can cause inflammatory response, oxidative stress and immune response, resulting in physiological disorders, structural damage and dysfunction, and ultimately changing the normal biological function of animals [5, 9, 10].

Moreover, the reproductive toxicity of MPs in animals and humans have been increasingly explored [11–13]. For example, some studies have revealed that MPs can significantly reduce the sperm quality and testosterone level of male mice [12]. The number of sperm that survives after exposure to MPs is dramatically decreased, and the rate of sperm malformation is significantly raised. Additionally, all levels of the testis' sperm cells exhibit atrophy, shedding, and apoptosis [13, 14]. For female mice, MPs can increase the level of IL-6 and decrease the level of MDA in mouse ovaries, affect the differentiation and survival rate of oocytes, and also cause certain damage to the mitochondria of oocytes [11]. MPs can also penetrate the placental barrier and interfere with the development of offspring, causing reproductive and genotoxic effects [15, 16]. Strikingly, there are definite studies that show that MPs are able to be exposed to the placenta of animals [17, 18]. Recent studies have found various shapes of microplastic fragments in human placenta, becoming the first evidence for the existence of MPs in human placenta [19]. However, it is still unclear whether MPs has potential effects on the physiological function of the animal placenta and whether MPs can affect maternal pregnancy and embryonic development through the placenta. Importantly, the underlying mechanisms have not been reported.

In this study, we investigated the toxic effects of MPs on the placenta of pregnant mice in early pregnancy. We found that MPs could significantly reduce the growth and reproductive performance of pregnant mice. Additionally, MP caused oxidative stress, dysfunction and inflammation in the placenta of pregnant mice. We finally demonstrated that MPs-induced placental toxicity was mediated through GRP78/IRE1α/JNK axis mediated apoptosis and endoplasmic reticulum stress. The results provide a theoretical basis for the toxicity of MPs on embryonic development and placenta in early pregnancy, and also provide a potential therapeutic target for the prevention of developmental toxicity of MPs in pregnant animals.

Materials and methods

Chemicals and reagents

Unibead monodispersed polystyrene microspheres (6-1-0500) were purchased from Baseline, Tianjin, China. The polystyrene microspheres we purchased are suspensions (2.5% w/v, 10 ml) with 5 μm particle monomers. This particle size can be directly dissolved in water to prepare the specified concentration. The concentration of MPs was determined based on reproductive toxicity study in mice [11, 12]. Information on primary and secondary antibodies is provided in Supplemental Table 1.

Animals and treatments

The experimental mice were 7-week-old C57BL/6J female mice purchased from Huafukang Bioscience Co. Inc (Beijing, China). The mice were allowed to have access to water and feed freely, and the formal experiment started after 1 week of adaptation. Female mice were trained on gavage with water prior to mating to reduce the potential stress during pregnancy, and were mated overnight with males at a ratio of 2:1. Pregnant mice were housed separately after a vaginal plug was observed. The day when a copulation plug was found was designated gestation day (GD) 0.

The pregnant mice were grouped as follows ($n=8$): All pregnant mice were divided into 4 groups, namely control group, 25 mg/kg MPs group, 50 mg/kg MPs group and 100 mg/kg MPs group. Each group had 8 pregnant mice. The control group was intragastrically administrated with normal saline, and the other MPs treatment groups were intragastrically administrated with MPs of different concentrations. After screening the dosage and treatment time of MPs by pre-experiment, we finally decided to treat with 25, 50, and 100 mg/kg b.w. MPs for 14 days to ensure the effect of the experiment.

Pregnant mice were treated with different concentrations of MPs from the 1st to 14th day of gestation. Body

weight, daily weight gain, daily feed intake and daily water intake of pregnant mice were recorded during GD 1–14. At GD 14, mice were euthanized by cervical dislocation after retro-orbital blood collection. All experimental procedures were approved by Institutional Animal Care and Use Committee of China Agricultural University (NO.AW91012202-1-2).

Sample collection

The pregnant mice were sacrificed by cervical dislocation after orbital blood collection on GD14. The fetus and placenta in pregnant mice were quickly dissected and separated on ice, the number of fetuses was recorded, and the fetus and placenta were then weighed. 6 to 8 placentas were fixed using 4% paraformaldehyde and stored until further analysis of morphological characteristics, while other placentas were placed in a 1.5 mL centrifuge tube and stored at -80℃ for related gene and protein detection.

Histopathological analysis

The placentas fixed with 4% paraformaldehyde overnight were trimmed into appropriate size tissue blocks, dehydrated by gradient ethanol, transparently treated with xylene, and added with melted paraffin. After paraffin infiltration into the tissue blocks, the paraffin blocks were embedded, and the embedded tissues were cut into 5 μm sections on a slicer for H&E staining. After sealing the slices were observed under a microscope and photographed. By observing the distribution of inflammatory cell infiltration and necrotic foci in the section tissue under microscope, the effect of MPs on the inflammatory injury of placenta tissue was determined. In addition, the distribution of placenta regions (dccidua, junctional zone, labyrinth zone) were statistically calculated and analyzed by Image J software.

Immunofluorescence staining

All placental tissues were divided in half and fixed in paraffin with the cut side up. The Cryotome FSE cryostat (Leica Company, Germany) thickness was adjusted to 6 μm; the tissue was cut into 6 μm tissue sections and subsequently adhered to adhesive slides. The adhesive slides with tissues were fixed in acetone for 10 min, then taken out to dry, placed in a slicing box, and stored in a freezer at -80 ℃.

After blocking the placental sections with 5% goat serum in PBS for 1 h, the primary antibodies were incubated overnight at 4 °C and then washed three times in PBS. Subsequently, the sections were incubated with AlexaFluor 594 fluorescent secondary antibody in the dark for 1 h, followed by nuclear staining with nuclear dye Hoechst 33,342. Finally, the placental sections fixed with anti-fluorescence quenched mounting medium, and visualized and photographed under a fluorescence microscope. Placental apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP Nick end labeling (TUNEL) assay.

Determination of placental oxidative stress levels

Catalase (CAT), glutathione peroxidase (GSH-Px) activity, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), myeloperoxidase (MPO), total superoxide dismutase (T-SOD) and total antioxidant capacity (T-AOC) in the placenta were assayed by a commercial reagent kit from Jiancheng Bioengineering Research Institute (Nanjing, China). All determination steps were completed according to the kit instructions. The kit contains a specific reagent as a control for loading variation, the optical density values were measured by using the SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

Western-blot analysis

10 mg placental tissue from each sample was homogenized using a Bead Ruptor Elite multifunctional sample homogenizer, and subsequently 100 μL RIPA lysate was added to each sample, lysed on ice for 30 min, and the supernatant was removed after centrifugation at 4 °C. After that, samples were prepared by measuring the protein sample concentration using the bicinchoninic acid (BCA) method. Subsequently, total proteins were separated by 10% SDS-PAGE and transferred to PVDF membranes. The PVDF membranes were incubated for primary antibodies (1:1000) at 4℃ overnight and then incubated with an appropriate secondary antibody (1:2000) at 25℃ for 1 h. Finally, the protein bands were developed using an enhanced chemifluorescence kit (Huaxingbio, Beijing, China) and visualized using an ImageQuant LAS mini system (GE Healthcare). Band density was analyzed using the Image J software. GAPDH was used as the loading control. The information of the primary antibody are shown in Supplemental Table 1.

Total RNA extraction and quantitative real-time PCR (qRT-PCR)

The primer sequences are shown in Supplemental Table 1. Total RNA from placenta was extracted by the Trizol reagent (#RN0402, Aidlab Biotechnologies Co, Beijing, China). Sequentially, cDNA was synthesized using a reverse-transcribed cDNA kit (#11204ES03, Yeasen biotech Co, Ltd, Shanghai, China). Gene expression levels were measured using the ABI 7500 realtime PCR system. All the operation steps were completed according to the instructions of the manufacturer. The *Gapdh* genes were used as the endogenous control for normalization. The reverse-transcribed cDNA was diluted 16-fold for qRT-PCR (ABI 7500, Alameda, CA, USA). A qRT-PCR

kit (# PC6002) was purchased from Beijing Aidlab Biotechnologies Co., Ltd. (Beijing, China). The total reaction volume was 10 μL, including (1) 5 μL of 2 × SYBR qPCR Mix (Low ROX), (2) 1 μL of DNA Template, (3) 0.2 μL of Forward Primer (10 Mm), (4) 0.2 μL of Reverse Primer (10 μM), and (5) 3.6 μL of dd H_2O. The reaction was run for one cycle at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, and 60 °C for 34 s. The results were calculated and analysed using the $2^{-\Delta\Delta Ct}$ method. All primers were synthesized by Sangon Biotech Co, Ltd. (Shanghai, China).

Statistical analysis

All data were statistically analyzed using SPSS statistical software (Version 25.0). One-way ANOVA was used to test the significance of differences, and Duncan's method was used for comparison between multiple groups, $P<.05$ were considered statistically significant.

Results

Effects of microplastics on growth performance of pregnant mice

Pregnant mice were gavaged with different concentrations of MPs from GD1 to GD14 and sacrificed at GD14. The experimental operation process and treatment are the same as shown in Fig. 1A. To explore the effects of MPs on growth performance of pregnant mice, we determined the effects of MPs on body weights, average daily weight gain, average daily feed intake, and average water consumption intake during GD1-GD14 (Fig. 1B). Specifically, MPs at 50 and 100 mg/kg significantly reduced the body weight of pregnant mice on GD14 (Fig. 1C). In addition, as shown in Fig. 1D, different concentrations of MPs could significantly reduce the average daily gain of pregnant mice. However, MPs did not significantly affect the average daily feed intake and daily water intake of pregnant mice (Fig. 1E-F).

Effects of microplastics on organ index and reproductive performance in pregnant mice

The organ weights and organ indices of pregnant mice treated with MPs were significantly increased in liver and spleen, but not in kidney (Fig. 2A-F). On the other hand, we also determined the effect of MPs on the reproductive performance of pregnant mice, and our results showed that: Compared with the control group, the fetal and placental weights of pregnant mice at higher MPs doses (50 mg/kg and 100 mg/kg) were significantly reduced ($P<.05$), and the total number of fetuses in each group showed a trend of gradually decreasing with the increase of MPs concentration, but there was no significant difference among the groups. Representative pictures of the number of fetal mice, individual fetal mice and placentas are shown in Fig. 2M-N.

Effects of microplastics on placental oxidative stress in pregnant mice

We further determined the effect of MPs on placental oxidative stress in pregnant mice. As shown in Fig. 3, the levels of MDA, CAT, MOP and H_2O_2 in the placenta of pregnant mice treated with high concentration of MPs were significantly increased, while the levels of T-AOC and SOD were significantly decreased. This suggests that high concentrations of MPs are able to induce oxidative stress in the placenta.

Effects of microplastics on placental inflammatory response in pregnant mice

The occurrence of oxidative stress in the placenta can further promote the inflammatory response of the placenta. Therefore, we further examined whether MPs could induce the occurrence of placental inflammatory response. For this purpose, we determined the effect of MPs on the mRNA expression of inflammatory factors in placental tissue from pregnant mice. The results showed that the addition of MPs significantly promoted the mRNA expression of inflammatory factors *Il-1α*, *Il-6* and *Tnf-α*, indicating that MPs induced inflammatory response in the placenta ($P<.05$). Consistent with the above results, we showed that with the increase of MPs treatment concentration, obvious inflammatory cell infiltration and necrotic foci appeared in the placental tissue of pregnant mice by analysis of H&E stained sections (Fig. 4C).

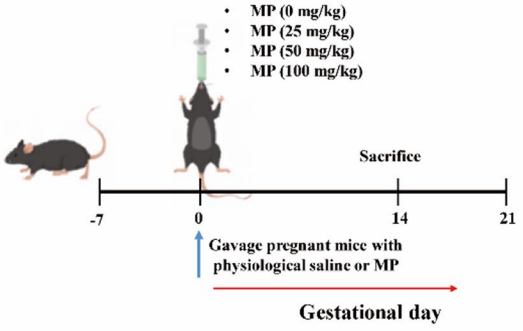
Effects of microplastics on placental nutrient transport in pregnant mice

The placental inflammatory response induced by MPs may affect placental nutrient transport, so we further examined the effect of MPs on placental nutrient transport in pregnant mice. For amino acid transporters, placental expression of *Slc36a1*, *Slc36a4*, and *Slc38a1* was significantly decreased in pregnant mice exposed to MPs, while MPs had no significant effect on neutral amino acid transporters such as *Slc38a2*, *Slc38a2*, and *Slc6a14*. In addition, MPs also significantly reduced the mRNA expression of fatty acid transporter *Slc27a4* and glucose transporter *Slc2a1* in the placenta of pregnant mice ($P<.05$).

Effect of microplastics on placental development and angiogenesis in pregnant mice

We further determined the effect of MPs on placental development in pregnant mice, and the results showed that treatment with MPs could significantly reduce the proportion of placental diffuse layer. In addition, we found that MPs could significantly reduce the mRNA expression of *Vegf*, *Vegfr* and *Atp1a1* in placental tissues of pregnant mice, indicating that MPs could significantly

A



B

Item	Groups				SEM	*P-value*
	0 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg		
Body weights , g						
G 0 , g	20.27	20.58	20.47	20.35	0.196	0.718
G 14 , g	29.43^{a}	28.34^{a}	27.45^{ab}	27.02^{b}	0.308	<0.01
Average daily weight gain, g/d	0.65^{a}	0.55^{b}	0.50^{b}	0.48^{b}	0.023	<0.01
Average daily feed intake, g/d	4.00	3.92	3.94	3.83	0.120	0.781
Average water consumption, g/d	6.19	6.08	6.31	6.11	0.162	0.767

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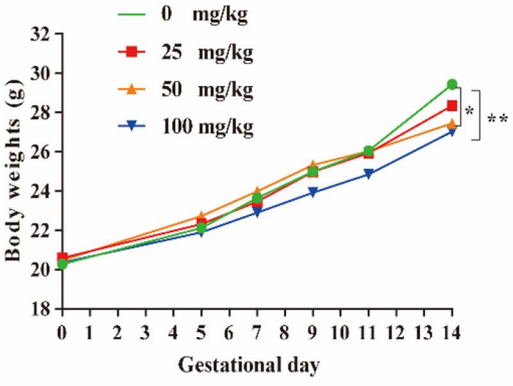

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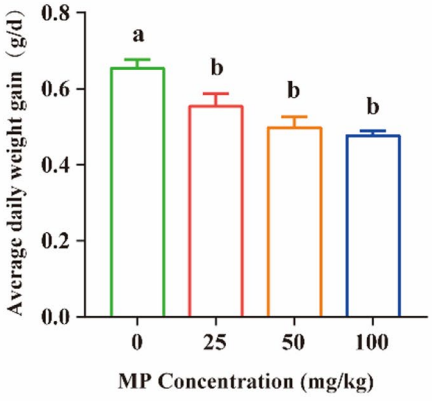

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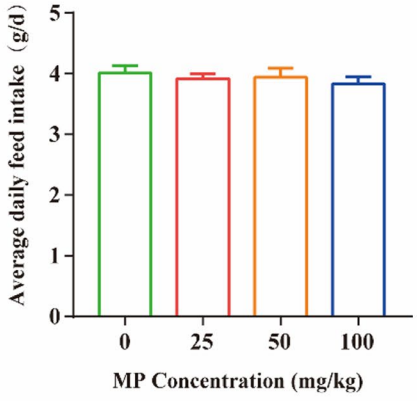

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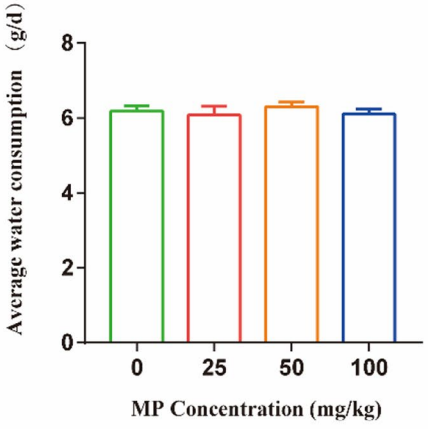

Fig. 1 Effects of microplastics on growth performance of pregnant mice. (**A**) Group assignments and experimental operation procedures (**B**) The table shows the effects of microplastics on the growth performance of pregnant mice (**C**-**F**) Bar plots of body weight, average daily gain, average daily feed intake, and average water comsumption of pregnant mice. Different asterisks represent significant differences ($_{***}$ $P < .001$, $_{**}$ $P < .01$, and $_{*}$ $P < .05$). Letters a-b indicate significant differences ($P < .05$)

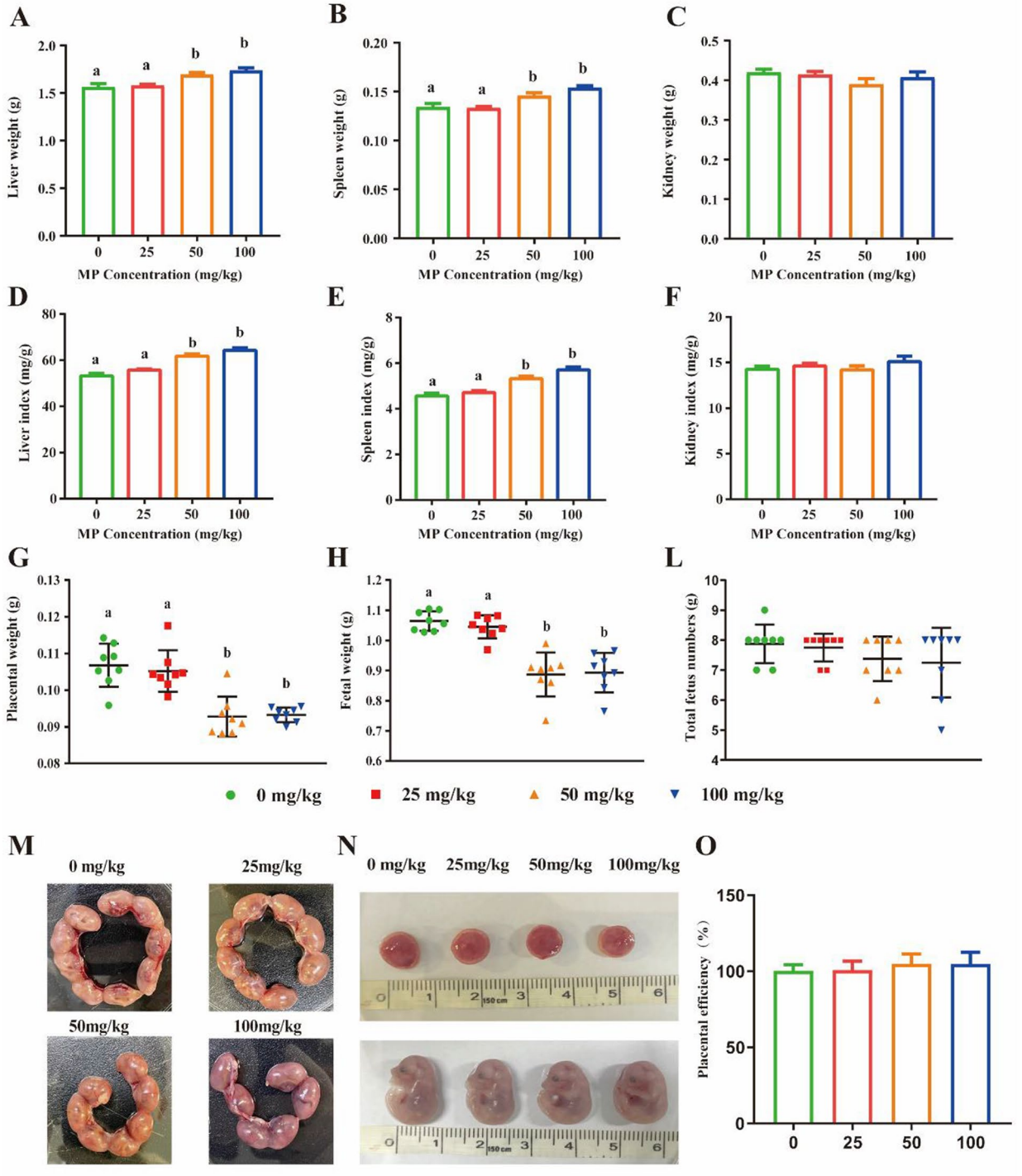

Fig. 2 Effects of microplastics on organ index and reproductive performance in pregnant mice. (**A**-**F**) Weights and organ index of liver, spleen, and kidney in pregnant mice. (**G**-**L**) Placental weight, fetal weight, and total number of fetuses in pregnant mice. (**M**-**N**) Representative images of the placenta and fetus of a pregnant mice. (**O**) Placental efficiency in pregnant mice. Letters a-b indicate significant differences ($P < .05$)

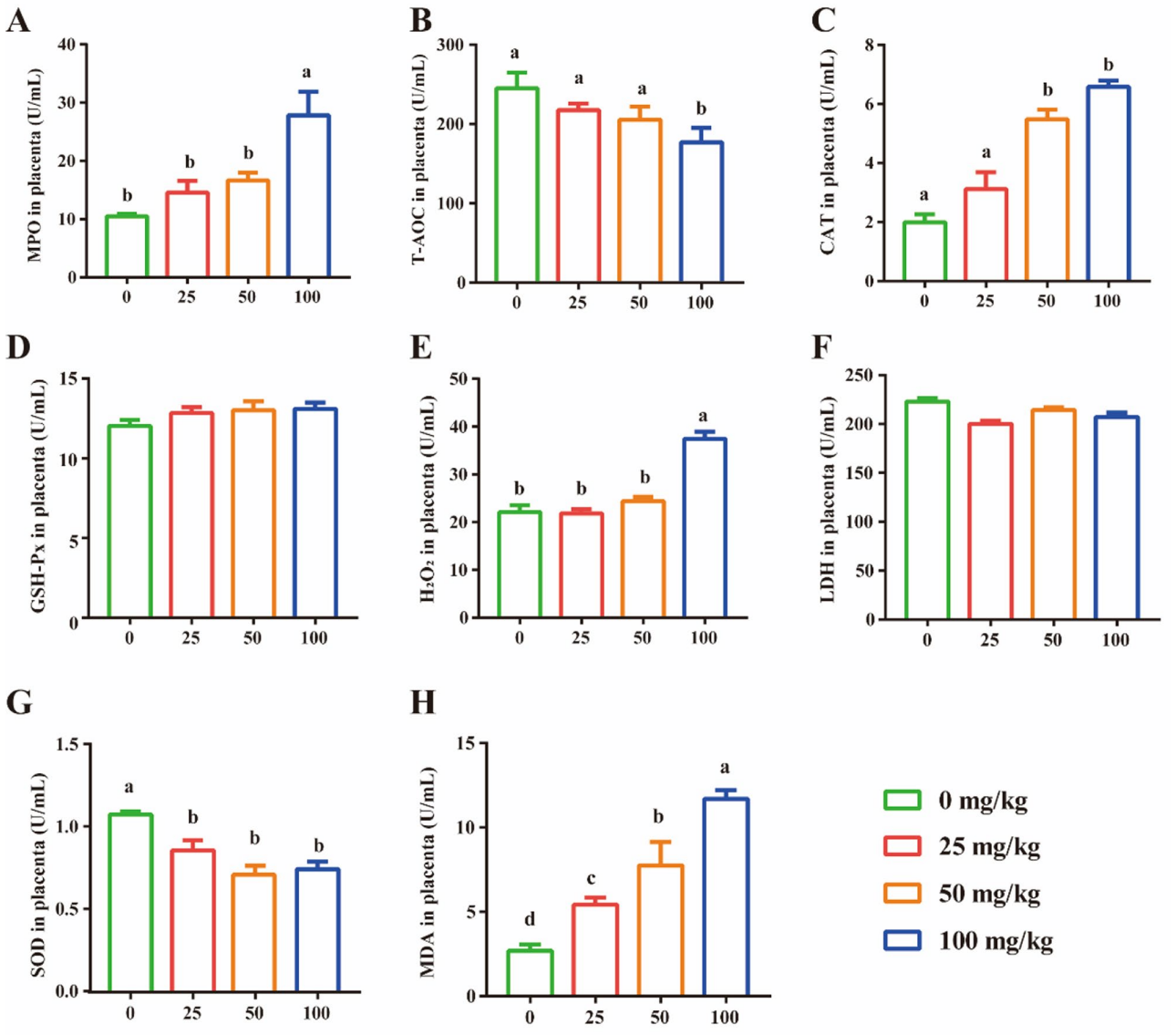

Fig. 3 Effects of microplastics on placental oxidative stress in pregnant mice. (**A**-**H**) Effects of different concentrations of microplastics on MPO, T-AOC, CAT, GSH-Px, H_2O_2, LDH MDA and SOD in placenta of pregnant mice respectively. Letters a-d indicate significant differences ($P < .05$)

inhibit placental development and angiogenesis in pregnant mice (Fig. 6).

Effects of microplastics on placental barrier function in pregnant mice

Western blot analysis showed that the expression levels of ZO-1, ZO-2, Claudin-1 and Claudin-3 were significantly decreased in the placenta of pregnant mice after MPs treatment, indicating that MPs could cause placental barrier dysfunction (Fig. 7A-B). Meanwhile, we further analyzed the expression of ZO-1 and ZO-2 in placental tissues by immunofluorescence staining, and the results also showed that with the increase of MPs concentration, the protein expression of ZO-1 and ZO-2 decreased, and the fluorescence intensity gradually weakened (Fig. 7C-D).

Effects of microplastics on placental apoptosis in pregnant mice

We further analyzed the effect of MPs on the expression of apoptosis related proteins in the placenta of pregnant mice by Western blot. The results showed that MPs significantly increased the expression of pro-apoptotic proteins Bax and cleaved-caspased-3 and decreased the expression of anti-apoptotic proteins Bcl-2 and Bcl-xl, indicating that MPs could induce apoptosis in the placenta of pregnant mice (Fig. 8A-B). We further showed through TUNEL staining analysis that TUNEL staining gradually deepened with the increase of MPs treatment

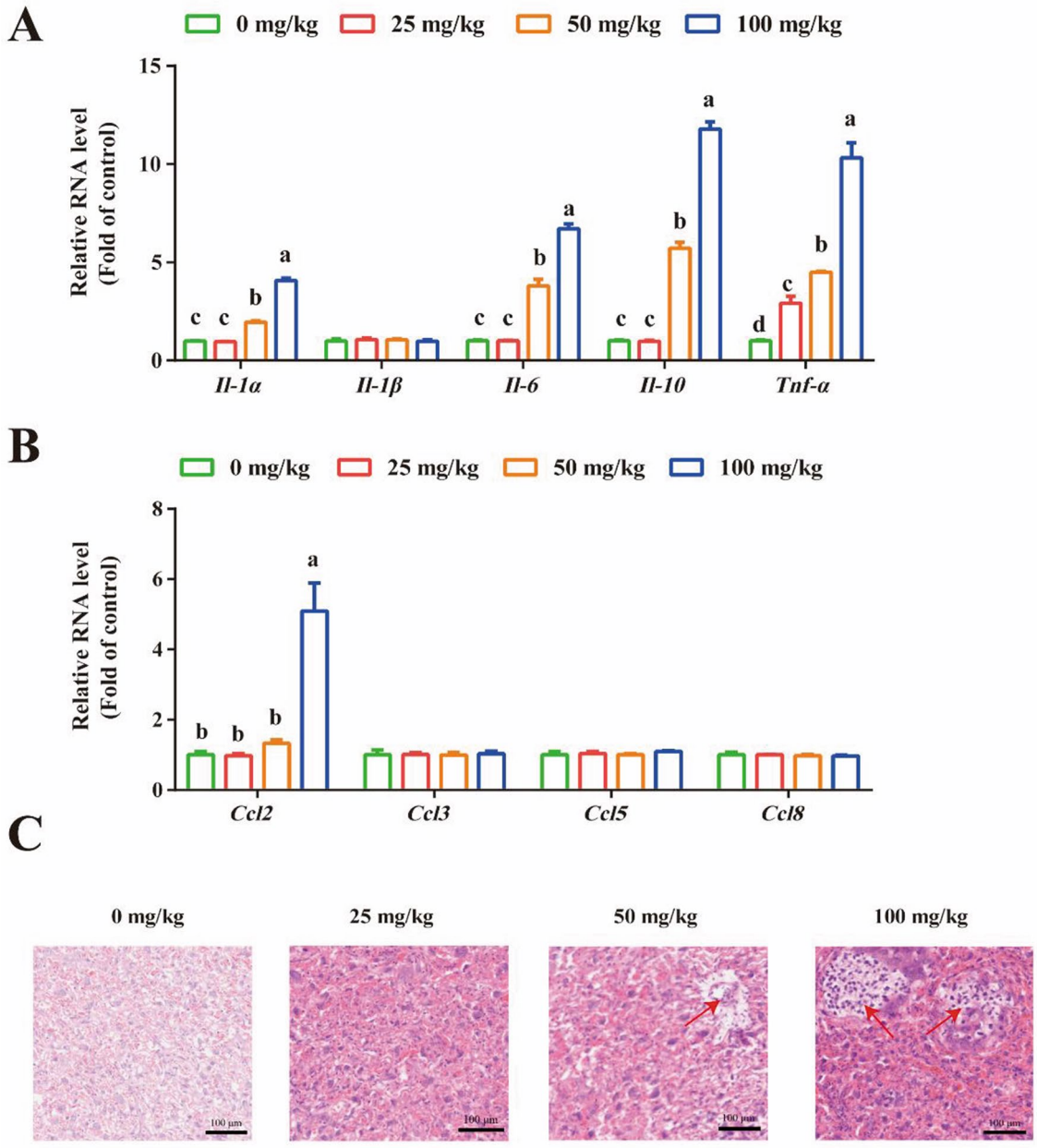

Fig. 4 Effects of microplastics on placental inflammation in pregnant mice. (**A**-**B**) Analysis of mRNA expression levels of inflammatory cytokines and chemokines in placenta of pregnant mice. (**C**) H&E stained section of placental tissue (Labyrinth zone) with a scale of 100 μm. The red arrows show necrotic foci. Histopathology and morphometry of the placental tissue performed by a blinded scorer. Letters a-d indicate significant differences ($P < .05$)

concentration, which also indicated that MPs could induce placental apoptosis after exposure to pregnant mice (Fig. 8C).

Microplastics induce placental endoplasmic reticulum stress and related mechanisms in pregnant mice

In order to further clarify the mechanism of placental dysfunction induced by MPs, the results showed that MPs induced endoplasmic reticulum (ER) stress in placentas, as indicated by the significant increase of endoplasmic reticulum marker protein GRP78. In addition, we further investigated the proteins involved in ER stress signaling pathway and found that MPs-induced ER stress was mediated by activation of p-IRE1α signaling (Fig. 9A-B). Subsequently, we measured the expression of downstream signaling proteins such as p-JNK and p-p65, and the results showed that IRE1α signaling activated the downstream p-JNK and p-p65, which was also consistent with the results of MPS-induced apoptosis and inflammatory response (Fig. 9C-D).

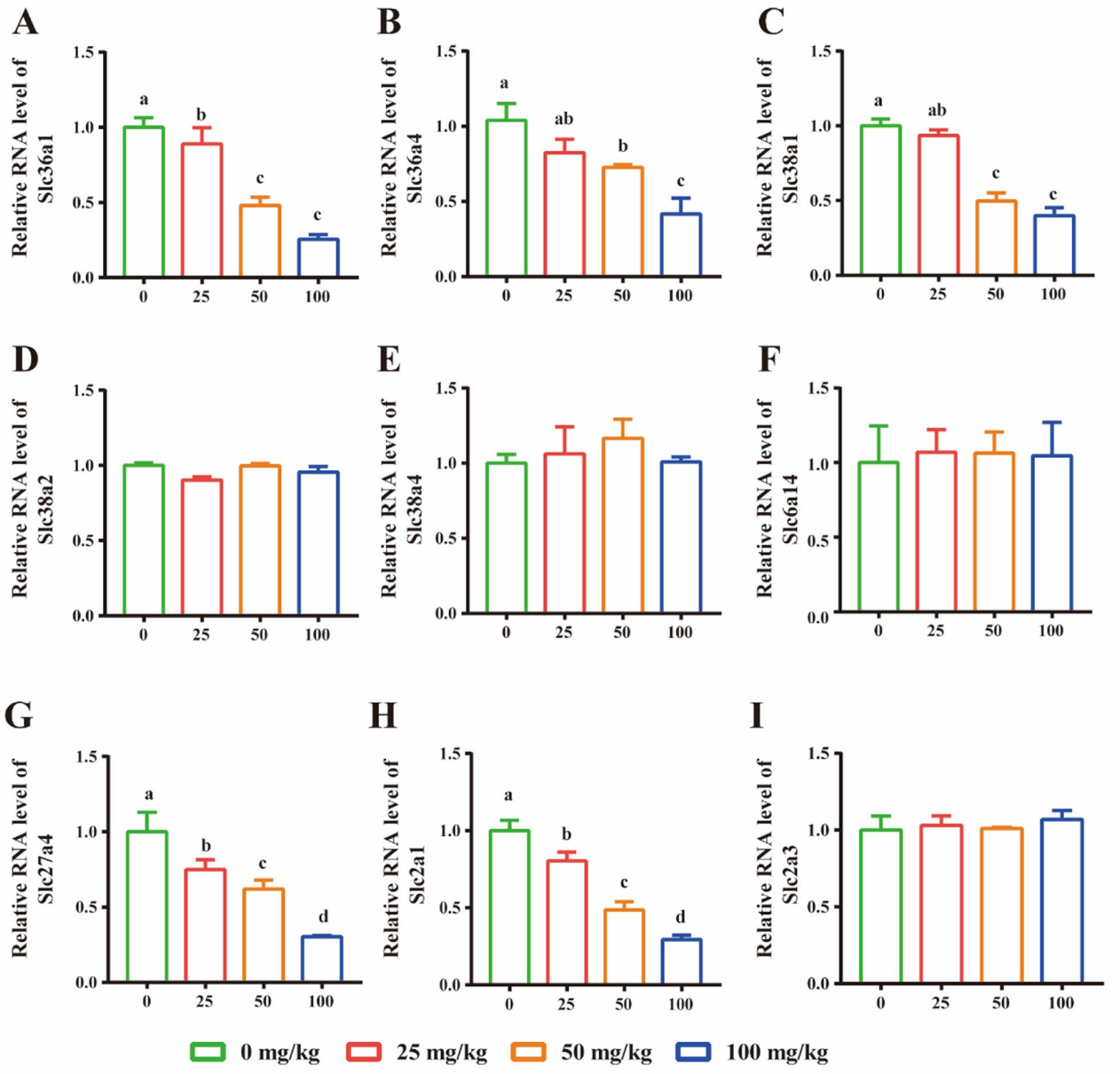

Fig. 5 Effects of microplastics on nutrient transport in placenta of pregnant mice. (**A**-**F**) Determination of mRNA expression of amino acid transporters in placentas. (**G**) Determination of mRNA expression of fatty acid transporters in placentas. (**H**-**I**) Determination of mRNA expression of glucose transporters in placentas. Letters a-d indicate significant differences ($P < .05$)

Discussion

MPs are mainly derived from plastic production and plastic products [20]. Under the action of physical and chemical factors and mechanical external forces, plastic products are degraded into MPs and distributed in the environment, resulting in environmental pollution [21]. Additionally, MPs are polymer chemicals with small particle size, large specific surface area, and difficult to degrade, so they can exist in our environment for a long time [6]. The United Nations Environment Program (UNEP) has identified microplastic pollution as one of the top ten new environmental pollution problems in the world [22]. Currently, MPs is a new type of global environmental pollutants, and its potential harm to humans and animals has attracted increasing attention [23]. Studies have shown that MPs can accumulate in the heart, liver, spleen, lung, kidney, testis and other organs through blood circulation, causing a series of toxic effects such as inflammatory response, oxidative stress, immune damage and metabolic disorder in animals [24–27]. In addition, some studies have found that MPs can induce reproductive toxicity in pregnant mice, resulting in oxidative stress in the body [11], and some studies have shown the

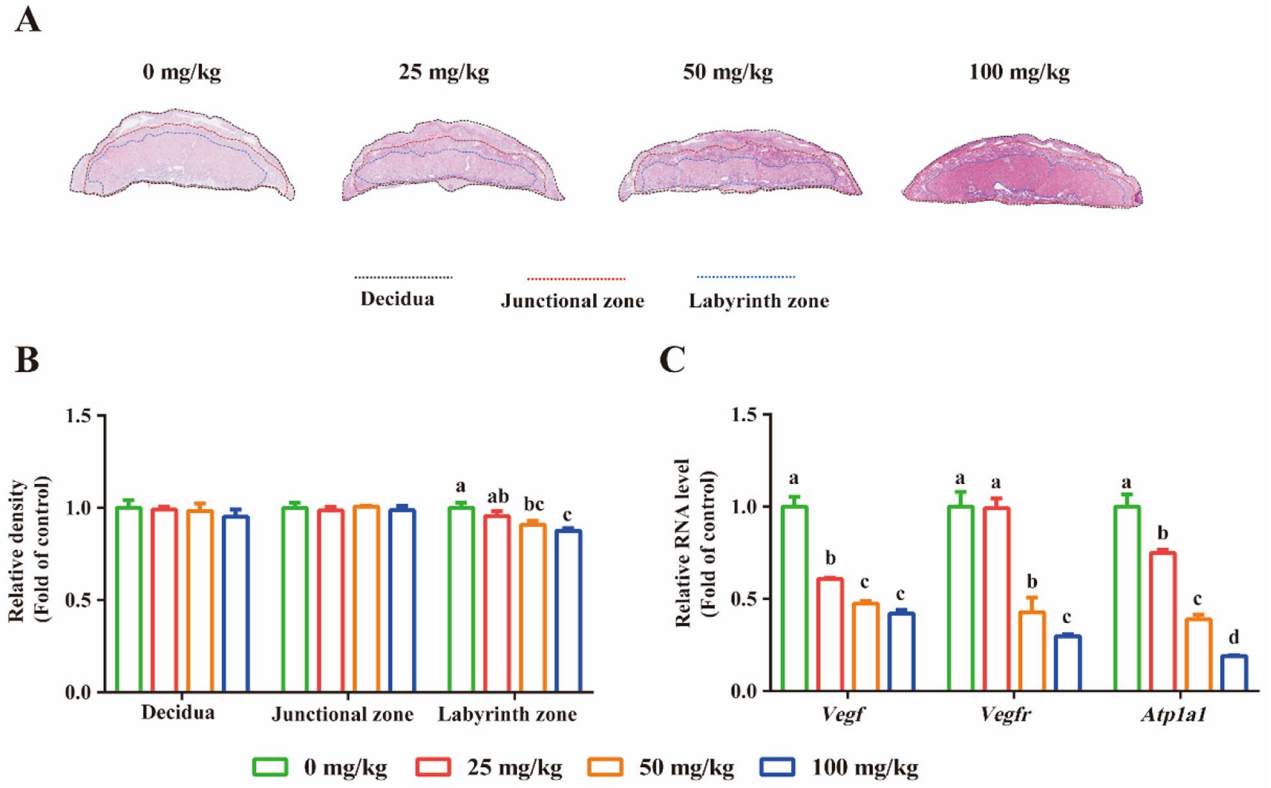

Fig. 6 Effects of microplastics on placental development and angiogenesis in pregnant mice. (**A**-**B**) Representative pictures and analysis of the development of different regions of the placenta (**C**) Analysis of mRNA expression of genes involved in vascular development in placentas. Letters a-d indicate significant differences ($P < .05$)

presence of microplastic particles in placental samples of subjects with successful pregnancies [28]. Also, some studies have found that MPs can induce reproductive toxicity in pregnant mice, leading to oxidative stress in the body [11], and some studies have shown that there are microplastic particles in placental samples of subjects with successful pregnancies [19], indicating that MPs can accumulate in the placenta. Studies have also found that exposure to MPs causes miscarriage in pregnant mice and is associated with impaired placental function [29, 30]. MPs exposure (50, 100, 150, or 200 μg/mL) increased oxidative stress, decreased mitochondrial membrane potential, and increased apoptosis in human trophoblast cells [31]. In addition, transcriptome analysis showed that MPs significantly interfered with cholesterol metabolism and the complement and coagulation cascade pathway in the placenta of pregnant mice [32]. However, whether the toxic effects of MPs on induced pregnancy are related to placental toxicity mediated by MPs remains unknown, and the mechanism of MPs toxicity to placenta needs further study. Therefore, we used pregnant mice as a model to explore the toxic effects and toxicological mechanism of MPs at different concentrations on placenta of pregnant mice. The aim of this study was to provide a theoretical basis for MPs mediated maternal developmental toxicity in pregnancy.

Growth performance is an important indicator to judge the health of maternal pregnant. In this study, we determined the effects of different concentrations of MPs on average daily gain, average daily feed intake and average water consumption of pregnant mice during GD 0–14. We found that MPs significantly reduced the average daily feed intake of pregnant mice, and also significantly reduced the body weight of pregnant mice at GD14. Similar reports have shown that a significant reduction in feed intake and the body weight in mice exposed to MPs of 4 μm and 10 μm. However, the mice treated with the size of 0.5 μm MPs had no significant effect on the growth performance of mice [12]. This result indicates that the harm of MPs to the animal body may be related to its particle size, and the particle size of MPs selected for our experiment is 5 μm, which is consistent with the results of the above research. Importantly, we further found that MPs at 50 mg/kg and 100 mg/kg could significantly reduce fetal and placental weights in pregnant mice. MPs have been reported to cause reproductive toxicity in animals, including oxidative stress in pregnant mice and decreased ovarian and oocyte quality in mice [11]. In addition, MPs can also cross the placental barrier

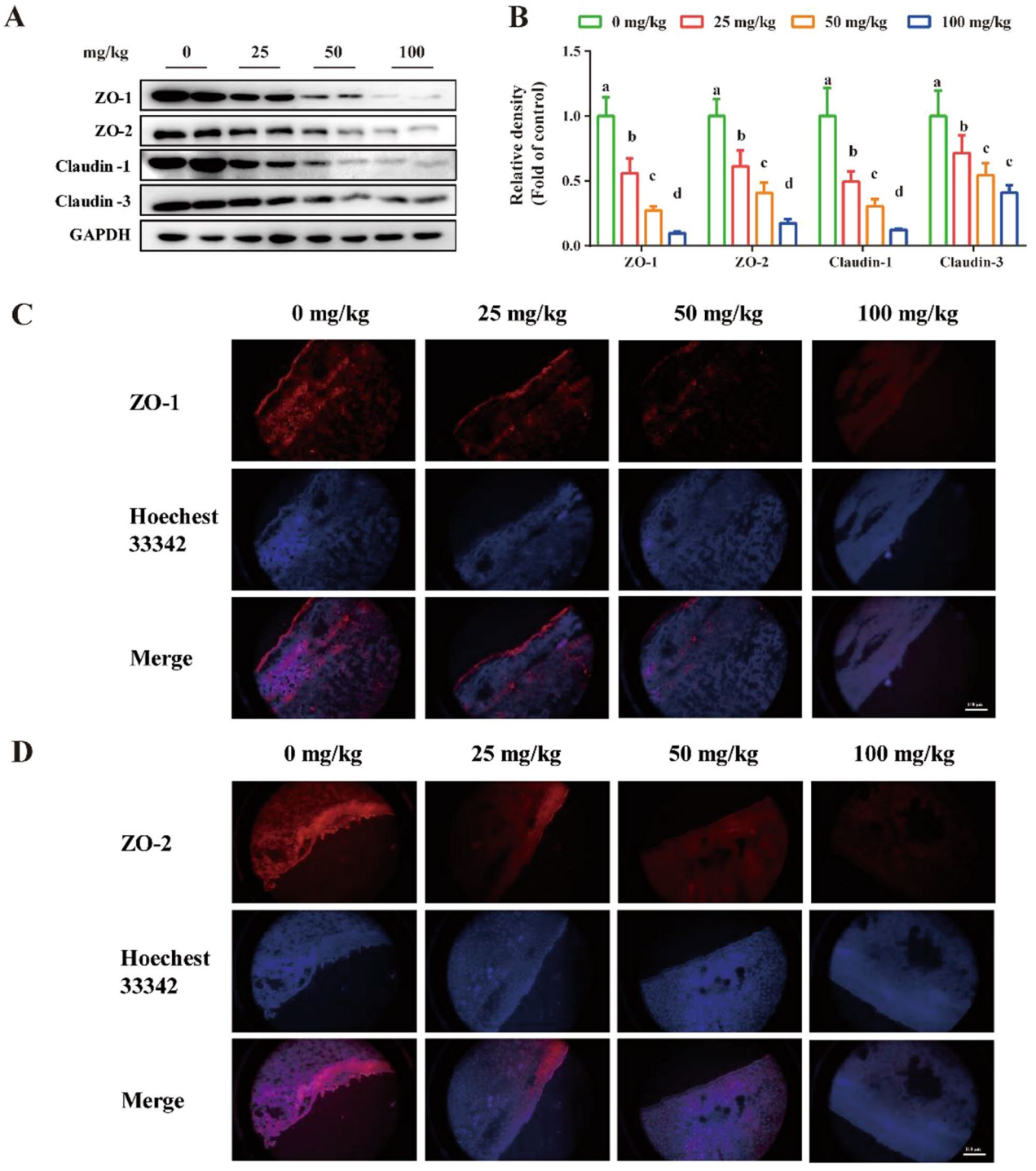

Fig. 7 Effects of microplastics on placental barrier function in pregnant mice. (**A**-**B**) Western blot was used to analyze the expression of proteins related to placental barrier function. $n = 3$ (**C**-**D**) Representative picture of immunofluorescence of ZO-1 and ZO-2 in placental tissue (Labyrinth zone). Letters a-d indicate significant differences ($P < .05$)

and deposit in placenta and fetal organs in pregnant rats, and reduce fetal and placental weight by 7% and 8%, respectively [33]. Taken together with the findings of our present trial, we speculate that the reduced reproductive performance of MPs in pregnant mice may be related to its potential toxicity to the placenta. Notably, recent studies have also demonstrated the presence of microplastic particles in human placenta tissues by scanning electron microscopy and transmission electron microscopy [18],

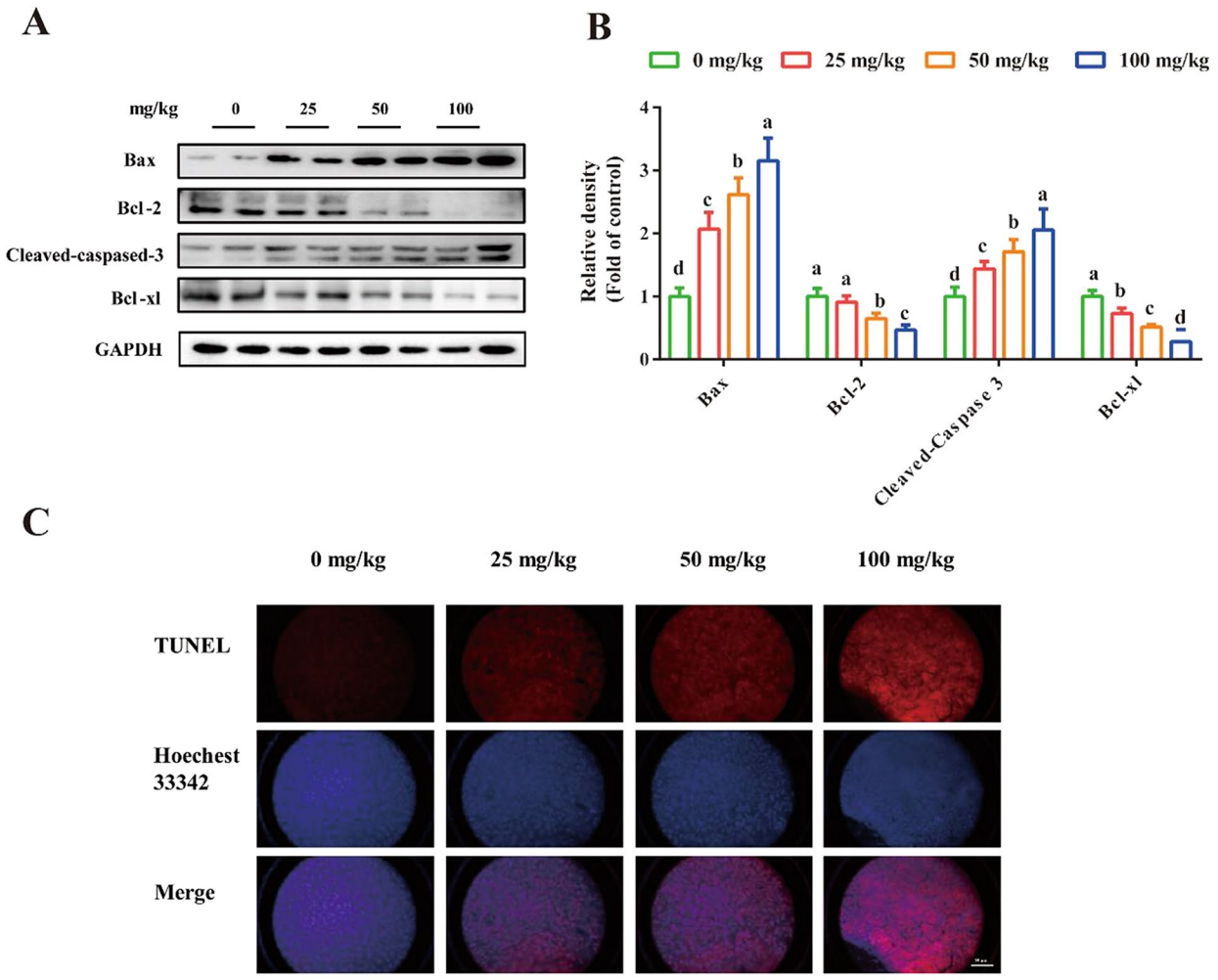

Fig. 8 Effects of microplastics on placental apoptosis in pregnant mice. (**A**-**B**) Western blot was used to analyze the expression of apoptosis related proteins in placental tissue. $n = 3$ (**C**) Representative picture of TUNEL staining of placental tissue (Labyrinth zone) with 50 μm scale bar. Letters a-d indicate significant differences ($P < .05$)

which further implied that MPs could affect the structure and function of the placenta.

Studies have shown that MPs can cause oxidative stress and cytotoxicity [34–36]. For instance, studies have found that MPs cause intestinal barrier dysfunction through ROS-mediated apoptosis of epithelial cells [34]. Moreover, it has been found that antioxidant enzymes can be activated after MPs exposure in different animal models, indicating the occurrence of oxidative stress [37–39]. In the present study, we found that MPs could significantly increase the levels of MDA, CAT, MPO and H_2O_2 in the placenta of pregnant mice, which also demonstrated that MPs could induce oxidative stress in the placenta. When the body is exposed to MPs, its antioxidant capacity will be depleted, leading to inflammatory response. In mice treated with MPs, the level of IL-1α was increased in serum, and chronic inflammatory cell infiltration was observed in the colon and duodenal lamina propria [26]. In addition, MPs could also cause significant reduction of spermatogenic cells, loose arrangement of cells and extensive shedding of spermatogenic cells in the mouse testis [12–14]. The mRNA levels of *Tnf-α*, *Il-6*, *Mcp-1* and *Cxcl10* in the mouse testis were significantly increased, indicating that MPs induced inflammatory response in the mouse testis [12]. In agree with above studies, MPs also activated the mRNA expression of inflammatory factors and chemokines in the placentas of pregnant mice. In addition, inflammatory cell infiltration and necrosis foci were observed in the placentas of pregnant mice treated with MPs.

The placenta is a place for material exchange between maternal and fetus during pregnancy and is responsible for providing all the nutrients needed for the growth of the fetus [40, 41]. At present, the convincing proof that

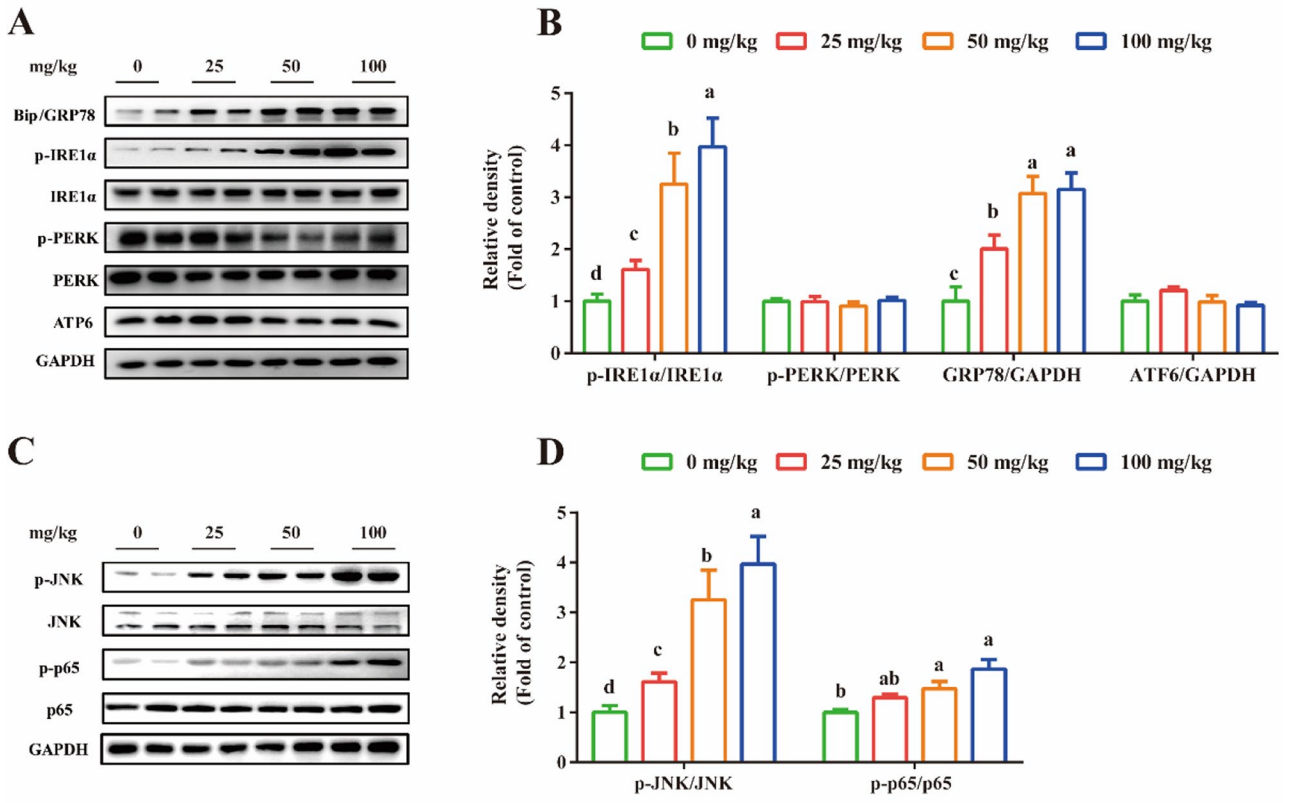

Fig. 9 Molecular mechanism of microplastics on placental tissue injury and dysfunction in pregnant mice. (**A**-**B**) Western blot was used to analyze the expression of endoplasmic reticulum stress-related proteins in placental tissues. $n = 3$ (**C**-**D**) Western blot was used to analyze the protein expression of JNK and p65 phosphorylation in placental tissue. Letters a-d indicate significant differences ($P < .05$)

MPs can produce reproductive toxicity in pregnant animals [42, 43]. Especially, the ability of MPs to cross the placental barrier has been demonstrated in an ex vivo placental perfusion model and a placental co-culture model [44–47]. More importantly, MPs were detected in placental tissue after delivery and in neonatal meconium, and their concentrations were even higher than those in adult feces, breast milk, and blood [19, 48, 49]. However, the specific effects of MPs on placental tissue are unknown. Subsequently, we determined that treatment with MPs was able to significantly inhibit the mRNA expression of some amino acid, fatty acid and glucose transporters in the placenta, indicating that MPs affected the placental nutrient transport function, which also explained the MPs-induced fetal weight loss in pregnant mice. Previous studies using an in vitro placental model found that 50 nm MPs could cross the placental barrier, indicating that MPs may enter the embryo through the placental barrier and affect the normal development of embryos [45]. Similarly, in aquatic organisms, it has been found that 0.6–1.0 μm MPs can cause non-lethal toxic effects such as cell proliferation and pericardial edema in zebrafish embryos, and even lethal effects on zebrafish embryos when the particle concentration reaches 250 mg/L [42]. On the other hand, an increasing body of evidence suggested that changes in the structure and function of the placenta can cause changes in pregnancy outcomes, and also affect the growth and development of the fetus [50, 51]. The early stages of placental development involve the development of the placental labyrinth zone, which consists of coiled epithelium between maternal blood sinuses and fetal blood vessels and is responsible for the exchange of nutrients, gases, and metabolic waste products between mother and fetus [52, 53]. In our study, we found that MPs can reduce the distribution of the labyrinth zone in the placenta, which is rich in capillaries. The reduction of the proportion of the labyrinth zone means that the maternal-fetal interface for material exchange is reduced, and the capacity for nutrient and oxygen exchange and metabolic waste clearance is also reduced [54]. In addition, MPs further impaired the barrier function of placental tissue in pregnant mice, which also increased the risk of invasion of harmful substances during pregnancy. These results suggest that MPs can induce reproductive toxicity in pregnant mice probably by affecting the structure and function of the placenta, which in turn affects the development of the fetus.

There is no doubt that many studies have demonstrated that MPs can induce apoptosis in the process of exerting its toxicity [55–57]. However, there are few reports on the toxic effects and toxic mechanisms of MPs on the placental tissue of pregnant animals, and whether MPs can cause apoptosis in the placental tissue remains to be explored. A recent report suggested that MPs could induce oxidative stress in human placental cells, leading to inflammation and apoptosis [58]. In line with the above reports, we successfully found that MPs could also induce placental apoptosis in animals modeled by pregnant mice. The endoplasmic reticulum is an important organelle for placental transport and metabolism. When the intracellular environment is in an unfavorable state, it can cause signal transduction from the endoplasmic reticulum to the cytoplasm and nucleus, eventually leading to adaptive survival or apoptosis. Currently, there are three specific pathways for endoplasmic reticulum stress (ERS): the protein kinase R-like ER kinase (PERK) pathway, inositol-requiring protein 1α (IRE1α) pathway, and activating transcription factor 6 (ATF6) pathway [59]. In this study, we determined that MPs are activated by ER stress via the IRE1α pathway. However, no significant change in ATF6 and PERK expression were observed. We hypothesized that placental tissue treated with MPs may be more dependent on IRE1α pathway in response to ER stress. Alternatively, PERK and ATF6 activation pathways may be less sensitive than IRE1α pathways under certain cell types or conditions. For example, IRE1α can trigger apoptosis by activating the downstream IRE1α-ASK1-JNK pathway through ER-mitochondria crosstalk in SiHa cells [60]. In addition, three ER stress-mediated apoptotic pathways have been reported, including the transcription factor CHOP/GADD153 pathway, Caspase-12 activation, and JNK activation [61]. Cell cycle, reproduction, apoptosis, and cell stress are just a few physiological and pathological processes that are affected by the JNK signal transduction pathway, a significant branch of the MAPK system [62, 63]. Our results showed that MPs treatment significantly activated JNK signaling pathway and induced the activation of pro-inflammatory signaling pathway NF-κB. These results further confirm that MPs mediate the generation of ER stress and apoptosis in placental tissues through the GRP78/IRE1α/JNK axis (Fig. 10).

Conclusion

In conclusion, we found that MPs could significantly induce fetal growth retardation and weight loss in pregnant mice. Importantly, we further show that MPs can induce placental toxicity and dysfunction in pregnant mice. Specifically, MPs caused oxidative stress and inflammation in the placental tissue of pregnant mice, reduced the nutrient transport capacity of the placental tissue, and damaged the placental barrier function. At the same time, MPs also caused apoptosis and endoplasmic reticulum stress in the placental tissue. In addition, we innovatively revealed that MPs mediated apoptosis

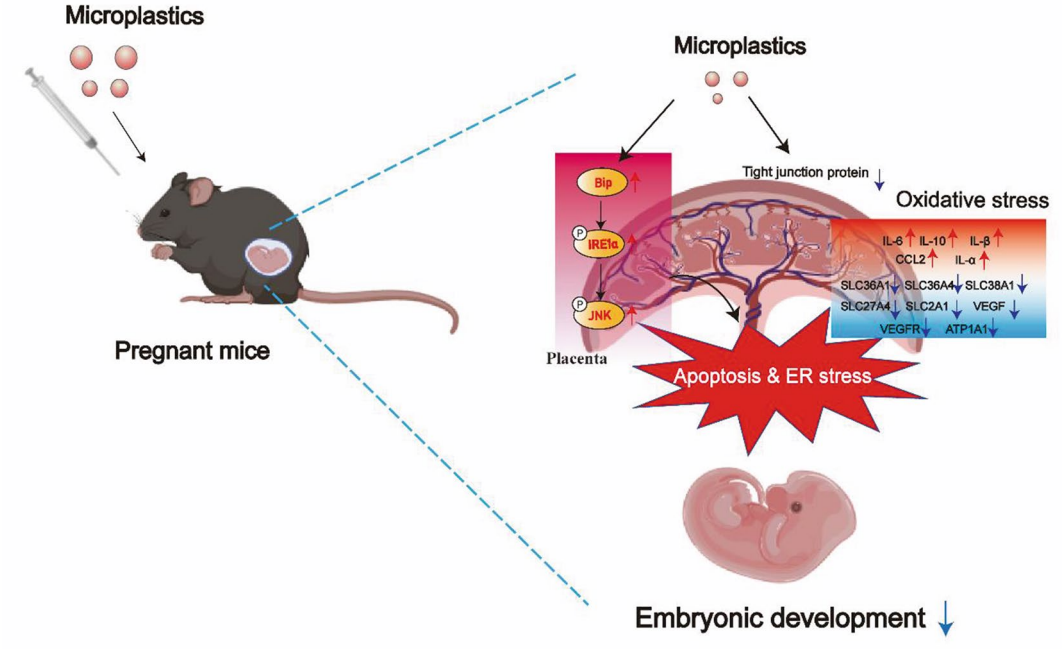

Fig. 10 Microplastics caused embryonic growth retardation and placental dysfunction in pregnant mice by activating GRP78/IRE1α/JNK axis induced apoptosis and endoplasmic reticulum stress

and endoplasmic reticulum stress in placental tissues of pregnant mice by activating GRP78/IRE1α/JNK axis. This may be the underlying mechanism of toxic effects of MPs on placental tissue. Our study provides a theoretical basis for the maternal developmental toxicity induced by MPs in pregnancy. At the same time, the toxic effects of MPs on the placenta of pregnant animals and the related mechanisms were first discovered, which will provide a therapeutic reference for alleviating the placental toxicity of MPs.

Abbreviations

CAT	Catalase
ER	Endoplasmic Reticulum
LDH	Lactate Dehydrogenase
H&E	Hematoxylin and Eosin
CAT	Catalase
GD	Gestation Day
GSH-Px	Glutathione Peroxidase
H_2O_2	Hydrogen Peroxide
MDA	Malondialdehyde
MPs	Microplastics
MPO	Myeloperoxidase
T-SOD	Total Superoxide Dismutase
T-AOC	Activity and Total Antioxidant Capacity

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12989-024-00595-5.

Supplementary Material 1

Author contributions

Zhenlong Wu designed the experiments. Jun Bai wrote the manuscript & completed the main experiments. Yuzeng Wang completed part of the experiments. Siwei Deng draw the figures and tables of manuscripts. Jun Bai collected samples and implicated in data analysis. Sheng Chen and Yang Ying implicated in manuscript editing. All the authors have read and approved the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

All experimental procedures were approved by Institutional Animal Care and Use Committee of China Agricultural University (NO.AW91012202-1-2). Related information is in supplementary file.

Competing interests

The authors declare no competing interests.

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