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New insights into the spleen injury by mitochondrial dysfunction of chicken under polystyrene microplastics stress

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ABSTRACT Microplastics biological toxicity, environmental persistence and biological chemicals have been paid widespread attention. Microplastics exposed to chicken spleen injury of the specific mechanism is unclear. Thus, we randomly assigned chickens to 4 groups: C (normal diet), L-MPs (1 mg/L), M-MPs (10 mg/L), and H-MPs (100 mg/L), and assessed spleen damage after 42 d of exposure. Morphologically, the boundary between the red and white pulp of the spleen was blurred, along with the expansion of the white pulp. It was further speculated that microplastics induced mitochondrial dynamic homeostasis (Drp1 upgraded, Mfn1, Mfn2, and OPA1 reduced), and provoked the mitochondrial apoptotic pathway (Bcl-2/Bax decreased, cytc, caspase3, and caspase9 raised), resulting in redox imbalance and lipid peroxide accumulation (MDA increased, CAT, GSH, and T-AOC plummeted), and further stimulated ferroptosis (FTH1, GPX4, and SLC7A11 decreased). Here we explored the impact of polystyrene microplastics on the spleen, as well as the programmed death (apoptosis and ferroptosis) involved, and the regulative role of mitochondria in this process. This could be of significant importance in bridging the gap in laboratory research on microplastics-induced spleen injury in chicken.

Key words: polystyrene microplastics, mitochondria, apoptosis, ferroptosis, spleen

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INTRODUCTION

Plastic brings great convenience to human life, followed by immeasurable pollution (Liu et al., 2023b; Wu et al., 2023). Tiny sheets of plastic between 100 nm and 5 mm in diameter are commonly defined as microplastics (**MP**) (Song et al., 2023). MPs pollution mainly comes from secondary release after fragmentation and degradation of daily plastic products, chemical products, and medical products such as masks (Hettiarachchi and Meegoda 2023; Kaur et al., 2023; Turner and Filella 2023). Some studies have pointed out the toxicity of polystyrene microplastics (**PS-MP**) to aquatic animals, such as the damage of 50 μm to the nervous system of goldfish larvae (Yang et al., 2020), and the metabolic disorder of liver in monopterus albus caused by 0.1 μm PS-MPs (Zhu et al., 2023). In mammals, mice neuroinflammation and parkinsonian lesions occurred after MPs exposure (Yang et al., 2019; Lee et al., 2022), and the 5 μm size demonstrates disruption of the rat intestinal barrier, further attracting digestive disorders (Huang et al., 2023b). A total of 1 to 12 μm MPs particles can directly damage the wound surface or pass through the blood barrier of the body to reach the organs (Wu et al., 2022; Shi et al., 2023b). MPs negatively affect the cardiovascular system of chickens (Zhang et al., 2022b), and MPs interfere with the proper functioning of the liver by disrupting the digestive metabolism of the chicken intestine (Yin et al., 2023), and these toxicological effects have been frequently reported in recent years. Nowadays, reports on the negative effects of MPs on the biology are gradually increasing (Khan et al., 2023), and people's attention to the harm of MPs is increasing by degrees.

The spleen is an important secondary lymphoid organ of chicken, which is involved in humoral and cellular

Abbreviations: MPs, microplastics; Bcl-2, B-cell lymphoma-2; HE, hematoxylin and eosin; GSH, glutathione; MDA, malondialdehyde; PPI, protein-protein interaction; Mfn1, mitofusin1; OPA1, optic atrophy 1; caspase9, cysteinyl aspartate specific proteinase 9; GPX4, glutathione peroxidase 4; FTH1, ferritin heavy chain 1; CTL, cytotoxic T lymphocyte; FasL, factor related apoptosis ligand; FADD, Fas-associating protein with a novel death domain; System Xc-, cystine/glutamate antiporter system; PS-MPs, polystyrene microplastics; cytc, cytochrome c; CAT, catalase; T-AOC, total antioxidant capacity; qRT-PCR, real-time quantitative polymerase chain Reaction; Drp1, dynamin-related protein 1; Mfn2, mitofusin2; Bax, Bcl2-associated X; Fas, factor related apoptosis; SLC7A11, solute carrier family 7 Member 11; mPTP, mitochondrial permeability transition pore; NK, natural killer cell; DD, death receptor domain; DISC, death inducing signaling complex

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immunity (He et al., 2023; Shi et al., 2023a; Vali et al., 2023). As the bursa degenerates after sexual maturity, the assumed character of the spleen in chicken should not be underestimated. B cells in the bursa migrate to other tissues, such as blood, spleen, thymus, and bone marrow, among which spleen and blood are the main colonization sites of B cells after migration (Paramithiotis and Ratcliffe 1993; Chen et al., 2020). Therefore, the spleen can often assess the degree to which the body is affected by the toxicity of external substances (environmental pollution, bacterial toxins, and virus attacks). MPs were able to influence lymphocyte subsets in the spleen of maternal and offspring mice (Tang et al., 2022). Splenic toxicity of microplastics had also been reported in oryzias latipes (Zhu et al., 2020) and carassius (Wei et al., 2023), which data was absent on spleen in chicken. The specific mechanism of injury in it was worth exploring.

As dynamic biophysical systems, mitochondria play a decisive role in regulating energy metabolism and controlling cell survival (Chen et al., 2023; Hao et al., 2023). Mitochondrial damage is one of the common mechanisms under MPs exposure (Liu et al., 2022b; Zhang et al., 2022b) in which MPs accumulate, breaking the electronic respiratory chain and further bringing mitochondrial membrane damage (Das 2023). Under external stimuli, the cell death signal is transmitted to mitochondria, B-cell lymphoma-2 (**Bcl-2**) family mediates the opening of membrane permeability transition pore and the efflux of cytochrome c (**cytc**), which initiates the mitochondrial apoptosis pathway (Martinou and Youle 2011; Wang et al., 2021). Ferroptosis is an iron dependence of programmed cell death, in which mitochondria have been found to play a crucial part (Dong et al., 2023; Li et al., 2023c). Mitochondria participate in the regulation of the body's redox equilibrium. Oxidation-reduction imbalance and lipid oxidation accumulation ensue which are the core of ferroptosis (Li et al., 2024a; Li et al., 2024b). Therefore, the role played by mitochondria in ferroptosis and apoptosis deserves attention.

Although the severity of MPs pollution has received extensive attention, studies on chicken spleen only paid little attention. The key of the correlation between them in PS-MPs induced spleen injury remains to be investigated. This exploration focused more on the correlation of mitochondria between PS-MPs induced splenocyte apoptosis and ferroptosis. We aimed to probe the impact of MPs exposure on spleen and the specific mechanism by constructing the exposure model of chicken PS-MPs, and to provide some views on the content of chicken spleen injury under MPs exposure.

MATERIALS AND METHODS

Animal Model Construction

Experimental PS-MPs with a diameter of 5 μm was purchased from Tesulang Chemical Company (China), and PS-MPs were exposed by water, and a homogeneous suspension was prepared for feeding using an ultrasonic machine, and the residual liquid of the tank was stirred every 2 h after addition to the tank to ensure that microplastic precipitation was minimized. A total of 60 one-day-old Ross 308 broilers (males: females = 1:1) purchased from Harbin Weiwei Co., LTD, were randomly divided by 4 experimental groups: control group C, L-MPs (PS-MPs 1 mg/L), M-MPs (PS-MPs 10 mg/L), and H-MPs (PS-MPs 100 mg/L), with 15 chickens in each group. One mg/L MPs was considered an ambient concentration (Das et al., 2023). The rearing conditions of 1-day-old chicks were maintained at 33°C, 65% humidity, and 16h sunshine to acclimate to the new environment. Subsequently, the temperature was decreased by 1°C every 3 d, and the humidity was decreased by 5% every week, finally maintained at 22°C, 55% humidity, and 16h sunshine (Li et al., 2023b). Chickens were exposed continuously for 42 d. The sinks were washed daily to reduce residue. Afterwards we euthanized the chickens and separated the spleen for subsequent experiments. This work was approved by the Institutional Animal Care and Use Committee of Northeast Forestry University (Approval No. UT-31, June 20, 2014).

Histopathological Observation

Fresh spleen tissues using paraformaldehyde fixed, embedded in paraffin, and cut the organization block into thin slices of 5 μm, and then by xylene elution paraffin, using ethanol for hydration. Fixed the slices on the glass slide and stained with hematoxylin and eosin (**H&E**) (Qing et al., 2022). Finally, we observed the pathological damage of the spleen using an optical microscope (Nikon DS-F12, Japan).

Transmission Electron Microscopy Observation

Prepared spleen into 1mm^3 slices and immediately fixed with 2.5% glutaraldehyde for 2h, 1% osmic acid for 2h, gradient dehydration with 50 to 100% ethanol, dehydration with acetone twice, and finally stained with uranyl acetate and led citrate for observation using transmission electron microscopy (JEM-1200EX, Japan).

Biochemical Analysis

We followed the instructions of catalase (**CAT**) (Nanjing Jiancheng, China), trace reduced glutathione (**GSH**) (Nanjing Jiancheng, China), total antioxidant capacity (**T-AOC**) (Wanlei bio, China), malondialdehyde (**MDA**) (Wanlei bio, China), and tissue iron (Nanjing Jiancheng, China) kits to detect the levels of CAT, GSH, T-AOC, MDA, and tissue iron in spleen.

Table 1. The primers involved in this study.

Gene	Forward (5′−3′)	Reverse (5′−3′)	Serial number
β-actin	GCCCTCTTCCAGCCATCTTT	AGTGTACAGGTAGCCCCTCC	NM_205518.2
Bax	TATGGGACACCAGGAGGGTA	CGTAGACCTTGCGGATAAAGC	XM_040676625.2
Bcl-2	ATCGTCGCCTTCTTCGAGTT	ATCCCATCCTCCGTTGTTCT	NM_205339.3
caspase9	CCGAAGGAGCAAGCACG	AGGTTGGACTGGGATGGAC	XM_046931415.1
caspase3	ATTCTACTGCTCCAGGCTACTACTCC	GTTCCTTCAGCATCCTACACAGAGAC	XM_046915477.1
Fas	TGTGTGCAGAATGCAAGTCAAG	GTGGTGGGTCAGGTCAACAT	XM_046919845.1
caspase8	CCTCTTGGGCATGGCTA	TGCTGCTCACCTCTTGATT	NM_204592.4
cytc	CACTGTAAGCGGAGCGGC	TGGAAGATGCCAAGACCCAC	NM_001079478.2
GPX4	AGAATGTGCGCTCAGGCG	TCCACTTGATGGCATTCCCC	NM_204220.3
FTH1	TGCAGGACATCAAGAAACCGGA	CTGCTCATCCAGGTAGTGAGTC	NM_205086.2
SLC7A11	GGGCTTTGAAACACCCAGGA	CTGACACAGGCCAAAACGTG	XM_040670527.2

Real-Time Quantitative Polymerase Chain Reaction

Spleen tissues were weighed and total RNA was extracted using Triazole method. cDNA was obtained using a reverse transcription kit (Vazyme Biotechnology, China). The levels of mRNA expression were measured using the HiScript II Q RT SuperMix kit (Vazyme Biotechnology, China) via an ABI-7500 (Applied Biosystems, USA) instrument. β-actin was used as the reference gene, and calculated according to the $2^{-\Delta\Delta Ct}$ method (Lei et al., 2023). The primer sequences are shown in Table 1.

Western Blot

The organization of the extracted total protein by SDS-PAGE gel electrophoresis separation was transferred to the PVDF membranes. At room temperature, blocked the membrane, then used the primary antibody to incubate overnight and incubated with the corresponding HRP conjugated secondary antibody, 2h duration. Finally, images were acquired using Image Quant LAS4000 (General Electric, USA). The antibody information is shown in Table 2.

Statistics of Microplastics Related Literature

Entered "TS= (Microplastics* or Microplastic)" into Web of Science to get the research status of MPs. Entered "TS=((Microplastics* or Microplastic) and (bird or birds or Chicken or peacock or quail or raptor or crane or heron or duck or goose or dove or ostrich))" to obtain the research status of MPs. Data from 2013 to 2022 were extracted and ranked, where the bars represented the number of studies, the broken line values were the percentage of the number of studies the total number of studies in the corresponding year, with MPs in purple and MPs-birds in blue. The different species were compared by typing "TS= ((Microplastics* or Microplastic) and X)", where X is the corresponding species name.

Gene Enrichment and Protein-Protein Interaction Analysis

In order to systematically explore the interrelationships between genes and proteins involved in this experiment, we used the online website Metascape (http://metascape.org) and STRING (http://string-db.org/) to conduct gene enrichment analysis and PPI network mapping on the collected target genes and proteins. Bar graph colored by p-values for gene enrichment analysis.

Statistics Analysis

The experimental data were from at least 3 separate experiments. The experimental data were analyzed for significance by one-way ANOVA analysis using IBM SPSS Statistics 20 software and compared with the control group. Data results were expressed as mean ± SD. $P < 0.05$ indicates a statistically significant difference. (*, **, *** respectively represent $P < 0.05$, $P < 0.01$, and $P < 0.001$).

Table 2. The antibodies used in this study.

Antibody name	Dilution ratio	Resource	Article number
β-actin	1: 2,000	Abclonal,USA	AC006
Mfn1	1:500	Abclonal, USA	A21293
Mfn2	1:500	Abclonal, USA	A19678
OPA1	1:500	Abclonal, USA	A9833
Drp1	1:500	WanLei Biotechnology, China	WL03028
Bax	1: 500	WanLei Biotechnology, China	WL01637
Bcl-2	1: 500	WanLei Biotechnology, China	WL01556
caspase-9	1: 500	WanLei Biotechnology, China	WL03421
caspase-3	1: 500	WanLei Biotechnology, China	WL02117
Fas	1:1,000	Abclonal, USA	A0233
caspase8	1: 500	WanLei Biotechnology, China	WL03426
cytc	1: 1,000	Boster Biotechnology, China	A03529
GPX4	1:500	Boster Biotechnology, China	A02059-1
FTH1	1:500	Boster Biotechnology, China	BM4487
SLC7A11	1:500	Boster Biotechnology, China	BM5318

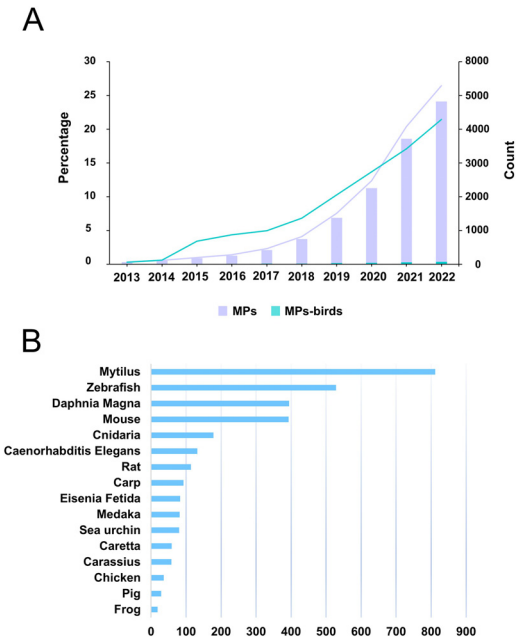

Figure 1. Investigation of MPs research status. (A) Research status of MPs and MPs-birds from 2013 to 2022. Bars showed the number of studies, the broken line values were the percentage of the number of studies the total number of studies in the corresponding year, with MPs in purple and MPs-birds in blue. (B) Bar graph of the status of research on microplastics in different species.

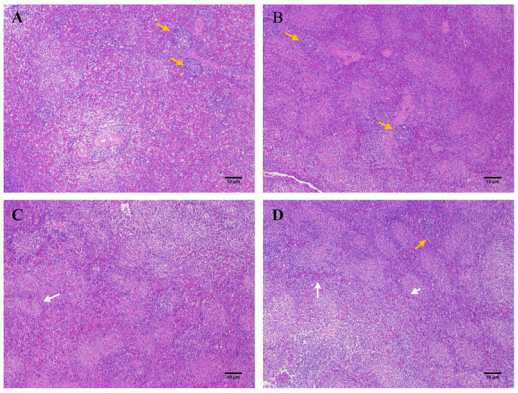

Figure 2. MPs led to mitochondrial damage. (A) Control group, clear red pulp and white pulp boundary, complete tissue structure. (B) L-MPS, exposed to 1mg/L, no obvious abnormality. (C) M-MPs, exposed to 10mg/L, the boundary between red and white pulp (white arrow) was blurred. (D) H-MPs, exposed to 100mg/L, the boundary between red and white pulp was confused (white arrow), and the number of splenic nodules were reduced (yellow arrow), in the meantime the white pulp was constantly expanded (H&E staining; magnification, 100×).

RESULTS

Investigation of MPs Research Status

We analyzed the MPs in all species research, attention to 18,240 articles were about MPs and only 3,446 articles were about MPs-birds. Although more and more studies had been conducted on MPs (Figure 1A), by analyzing the research status among species, we found that compared with other species, there is still a large gap in the research on chicken (Figure 1B).

MPs Promoted the Pathological Changes of Chicken Spleen

In the control group, the red and white pulp interface of the spleen was clear, the morphology was complete, the arrangement was close, and the structure of splenic nodules (yellow arrow) is clear (Figure 2A). After MPs treatment, no apparent damage was found in the L-MPs (Figure 2B). The spleen of the M-MPs and H-MPs showed abnormalities, the boundary between the red and white pulp was blurred (white arrow), and the number of splenic nodules were reduced (yellow arrow), in the meantime the white pulp was constantly expanded (Figures 2C and 2D). It was suggested that the exposure of MPs caused pathological damage to the spleen.

MPs Led to Mitochondrial Damage

By observing the damage of the spleen's ultrastructure through transmission electron microscopy, the typical symptoms of ferroptosis included increased mitochondrial membrane density and ridge rupture were found (Liang et al., 2023). With the increase of MPs exposure concentration, the mitochondrial damage became more pronounced. Increased mitochondrial membrane density and the cristae were broken in the M-MPs and even disappeared in the H-MPs (Figure 3A). The red arrows were diseased mitochondria. We measured the levels of proteins involved in mitochondrial dynamics. The levels of mitochondrial fusion and fission proteins corresponding to the exposed group showed abnormality: the fission protein dynamin-related protein 1 (**Drp1**) showed an increasing trend, and the fusion proteins optic atrophy 1 (**OPA1**), mitofusin 1 (**Mfn1**), and mitofusin 2 (**Mfn2**) showed a decreasing trend (Figure 3B and 3C). These manifestations may indicate that MPs elicited damage to the spleen mitochondrial structure and dynamic disorder.

MPs Caused Oxidation-Reduction Imbalance in Chicken Spleen

The oxidation regulation indicators CAT, GSH, MDA, and T-AOC were detected (Figures 4A and 4B). In the M-MPs and H-MPs, anti-oxidation index CAT activities, GSH, and T-AOC content decreased markedly ($p < 0.05-0.001$), MDA content significantly enhanced ($p < 0.05-0.001$). The results indicated that the spleen tissue was exposed to the oxidation-reduction imbalance under the MPs, and the body had oxidative stress.

MPs Provoked Apoptosis in Chicken Spleen

After exposure to MPs, apoptosis factors in spleen tissue changed significantly. The protein expression levels of Bcl2-associated X (**Bax**), cysteinyl aspartate specific proteinase 9 (**caspase 9**), caspase 3, and cytc in the

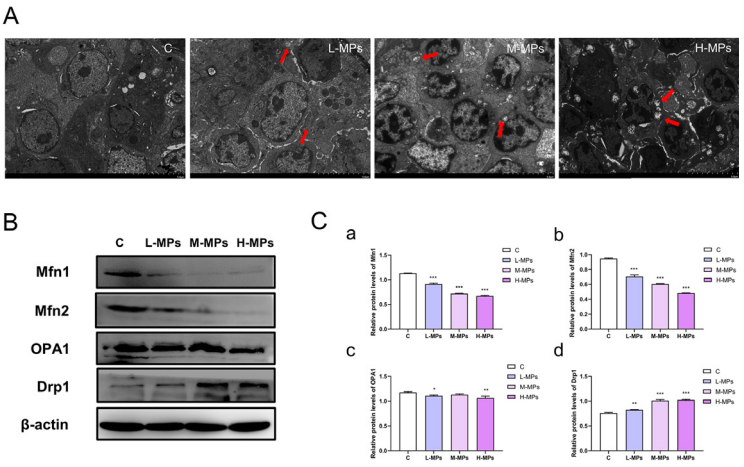

Figure 3. MPs led to mitochondrial damage. (A) MPs exposure spleen ultrastructure observation, mitochondrial membrane density was expanded, the red arrows indicate the mitochondria damage including mitochondrial crest broken and disappeared. (B) Western blot of mitochondria-associated proteins. (C) Quantitative analysis of Mfn1, Mfn2, OPA1, and Drp1 protein expression. Data were expressed as mean ± SD, n = 3. *, **, ***, denotes: $p < 0.05$, 0.01 and 0.001, respectively.

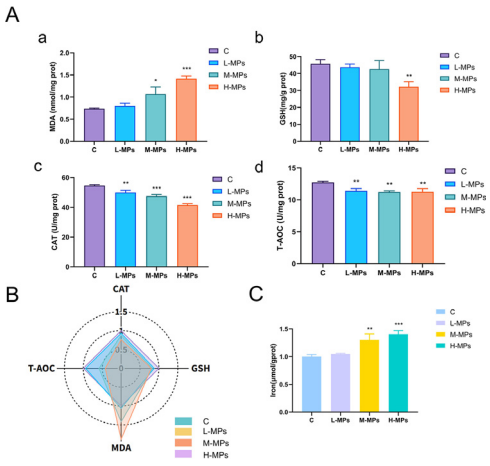

Figure 4. MPs caused oxidative stress in the spleen. (A and B) C, L-MPs, M-MPs, H-MPs corresponding oxidative stress indicators CAT, GSH, MDA, and T-AOC detection. (C) Determination of tissue iron content. Data were expressed as mean ± SD, n = 3. *, **, ***, denotes: $p < 0.05$, 0.01, and 0.001, respectively.

mitochondrial pathway of apoptosis in the MPs exposure groups were sensibly increased ($p < 0.001$), and Bcl-2 was significantly decreased ($p < 0.001$). Death receptor pathway factor: factor related apoptosis (**Fas**), and caspase 8 were significantly augmented ($p < 0.01$ −0.001) (Figures 5A and 5B). In addition, the mRNA levels of Bax, Bcl-2, caspase 9, caspase 3, cytc, Fas, and caspase 8 were measured (Figure 5C). The results suggested that MPs exposure triggered the spleen mitochondrial pathway and death receptor pathway of apoptosis, which may further cause spleen injury.

MPs Induced Ferroptosis in Chicken Spleen

To explore the MPs exposed the exact mechanism of spleen injury, ferroptosis-related factors were tested. MPs exposure significantly inhibited the protein expression levels of ferroptosis-related factors glutathione peroxidase 4 (**GPX4**), solute carrier family 7 member 11 (**SLC7A11**), and ferritin heavy chain 1 (**FTH1**) ($p < 0.001$), and the mRNA levels were basically consistent with the protein (Figure 6). Iron levels in tissues was also detected, and we found that the L-MPs group did not increase significantly after exposure to MPs, while the M-MPs and H-MPs groups increased significantly ($P < 0.001$) (Figure 3B). The results displayed that the exposure of MPs would induce ferroptosis in the spleen.

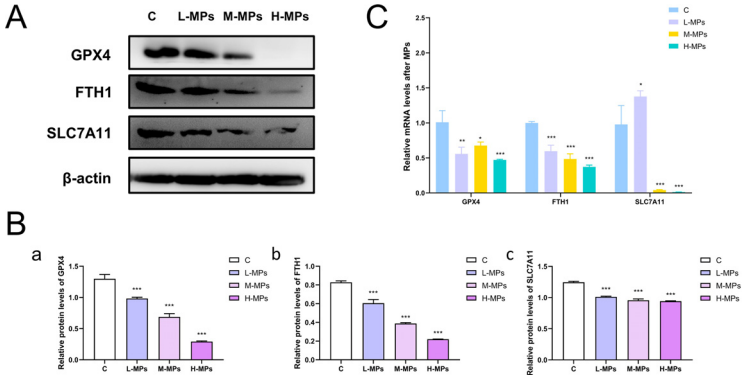

Figure 5. MPs induced ferroptosis in spleen. (A) Western Blot results in ferroptosis-related proteins. (B) Quantitative analysis of GPX4, FTH1, and SLC7A11 protein expression. (C) Ferroptosis-related factors of mRNA expression changes. Data were expressed as mean ± SD, n = 3. *, **, ***, denotes: $p < 0.05$, 0.01, and 0.001, respectively.

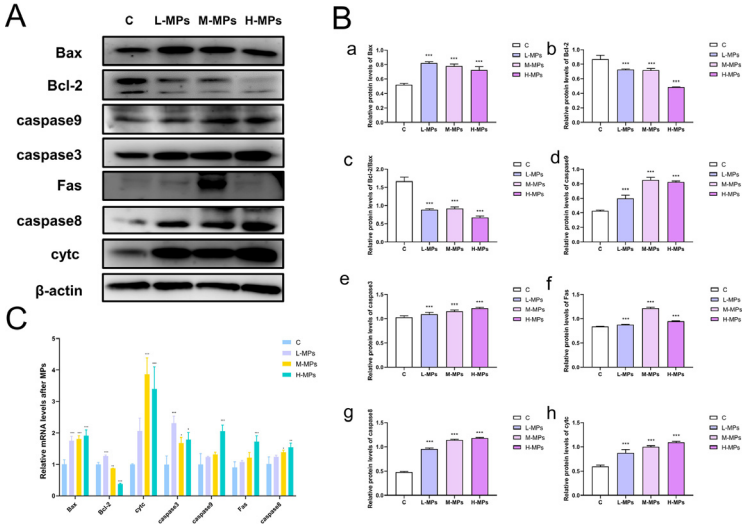

Figure 6. MPs provoked apoptosis in spleen. (A) Western Blot of proteins related to the mitochondrial pathway and death receptor pathway. (B) Quantitative analysis of protein expression. (C) Changes in mRNA expression of factors involved in the mitochondrial pathway and death receptor pathway. Data were expressed as mean ± SD, n = 3. *, **, ***, denotes: $p < 0.05$, 0.01, and 0.001, respectively.

Gene Enrichment and Protein Interaction Network Analysis

By establishing the protein-protein interaction network at the protein level, we discovered that the proteins related to apoptosis, mitochondrial damage and ferroptosis formed a dense network (Figure 7A). We used Metascape to perform enrichment analysis of the genes involved in this experiment, and the top 7 were screened, including apoptosis, mitochondrial fusion, and ferroptosis, which coincided with our experimental investigation (Figure 7B).

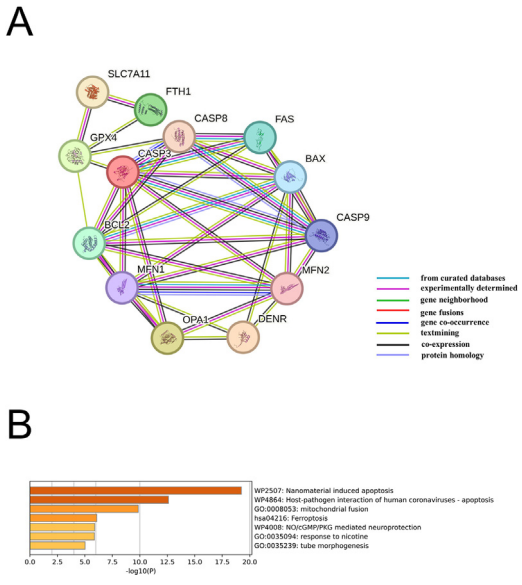

Figure 7. Gene enrichment and protein interaction network analysis. (A) Interaction network of apoptosis, mitochondrial damage, and ferroptosis proteins. Among them, DENR, CASP3, CASP9, and CASP8 represent Drp1, caspase 3, caspase 9, and caspase 8 respectively. (B) Differential gene enrichment analysis.

DISCUSSION

At present, MPs pollution has become a focus topic. The research related to MPs began to rise rapidly from 2019, and there was still an upward trend. From the perspective of time development, people's research on MPs in birds has been steadily increasing. But the lack of laboratory data on the damage caused by MPs exposure should not be overlooked (Yin et al., 2022; Li et al., 2023a). Chickens eat plastic products by mistake, which are further decomposed in the gizzard and then absorbed by the spleen (Rivers-Auty et al., 2023). As one of the largest meat consumption, MPs for its wide variety of risk, involving food and environment. Our focus was on chicken, and PS-MPs exposure models were constructed to examine the mechanism of spleen injury caused by concrete. For the first time, it was demonstrated that MPs cause chicken spleen injury by triggering mitochondrial structural and functional collapse, which triggers mitochondrial pathway apoptosis and subsequent redox imbalance and iron death.

Mitochondrial damage attracted by MPs exposure has been frequently reported (Zhang et al., 2022a; Gu et al., 2023). MPs significantly increased oxidation levels in oocytes and embryos, and produced mitochondrial dysfunction and apoptosis (Zhang et al., 2023). Based on our experiment, MPs treatment gave rise mitochondrial structural damage, and accompanied by the disturbance of dynamics (Figure 2), which was consistent with other MPs studies (Elsheikh et al., 2023). Apoptosis is a kind of programmed cell death, which can be divided into endogenous and exogenous (Ji et al., 2019). The endogenous includes the mitochondrial pathway and endoplasmic reticulum pathway, and the exogenous consists of death receptor pathway. As a member of the family of Bcl-2, pro-apoptotic proteins Bax generally exists in the cytoplasm. When the apoptotic signal is stimulated, it will locate on the surface of mitochondria, causing the

mitochondrial permeability transition pore (**mPTP**) to open, and increasing membrane permeability. With the release of cytc, caspase 9 and caspase3 and other apoptotic factors were released to cause mitochondrial pathway of apoptosis (Xu et al., 2023). In this research, the pro-apoptotic factor Bax was markedly expanded, while the anti-apoptotic factor Bcl-2 was memorably declined after MPs exposure (Figure 4). The release of cytc, and the activation of caspase family confirmed that MPs exposure activated apoptosis in spleen via the mitochondrial pathway.

Numerous studies have demonstrated that mitochondrial dynamics proteins (Mfn1, Mfn2, Drp1, and OPA1) are closely linked to apoptosis (Liu et al., 2023; Wang et al., 2023; Sun et al., 2024). When stimulated by death signals, Bax/Bak forms oligomers (Bax oligomerizes) to form pores in the outer mitochondrial membrane, releasing cytc and activating apoptosis. In rats, Mfn1 overexpression inhibited Bax translocation, oligomerization, and subsequent activation of apoptosis (Ryu et al., 2012; Campbell et al., 2019). After Mfn1/2 and OPA1 depletion (Wang et al., 2023), mitochondria were damaged and showed Bax activation and subsequent apoptosis, while cells with Drp1 silencing showed resistance to Bax activation. In this research, Mfn1, Mfn2, and OPA1 in M-MPs and H-MPs groups were significantly decreased, while Drp1 and Bax were visibly increased (Figures 2A and 4A). They speculate that MPs exposure elicited spleen cell apoptosis by inducing mitochondrial dynamics disorder, it may be due to the aberrant expression of Mfn1/2, OPA1, and Drp1, which disturbed Bax oligomerization and the localization of Bax in mitochondria, resulting in the activation of apoptosis in spleen cells.

Apoptosis in the progression of the immune system, and in the process of immune response plays a decisive role (Sobrido-Cameán and Barreiro-Iglesias 2018; Huang et al., 2023a). It affects the development of lymphocytes, participates in the exclusion of excessive immunocyte in the immune response, and also regulates the clearance of target cells by cytotoxic T lymphocyte (**CTL**) and natural killer cell (**NK**). CTL can induce apoptosis of target cells containing Fas on the surface by releasing factor related apoptosis ligand (**FasL**) to achieve the effect of eliminating target cells. The cell surface receptor Fas binds to the ligand FasL, forming a death receptor domain (**DD**) on the cytoplasmic side of Fas. It recruits procaspase 8 through the adaptor molecule Fas-associating protein with a novel death domain (**FADD**) to create a death inducing signaling complex (**DISC**), hydrolysis activates caspase 8, which in turn activates downstream caspase 3 to execute apoptosis (Huang et al., 2023a). The spleen is a typical immune organ. We detected the levels of Fas and caspase 8 protein in spleen cells and found that both proteins were significantly increased in M-MPs and H-MPs groups (Figures 4A and 4B). It indicated that in the spleen, MPs exposure may trigger the targeting effect of killer T cells to induce apoptosis through the death receptor pathway. During the study, the absence of dose-dependent spleen injury could be attributed to self-regulation. At low concentrations, the body resisted the damage of MPs by self-regulation, this was within the affordable range. Once this threshold was exceeded, the consequences aroused by the outside world cannot be reversed, which may be the reason why the high concentration damage is significant.

When mitochondria are damaged, redox imbalance and a large amount of ROS burst will follow. This work found that after MPs exposure, spleen redox imbalance, manifested by a significant decrease in antioxidant enzyme CAT activity, GSH and T-AOC content, likewise a significant increase in MDA, and the product of lipid peroxidation (Figure 3A). High levels of free radicals will attack intracellular lipids and proteins, damage their structure and function, then cause cell damage (Gong et al., 2022; Liu et al., 2022a; Liang et al., 2023). Physiologically, lipid peroxides can be removed, and cells take extracellular cystine into the cell to synthesize GSH through the cystine/glutamate antiporter system (**System Xc-**), which is consist of the light chain subunit SLC7A11 and the heavy chain subunit SLC3A2 (Pan et al., 2023; Zhou et al., 2023). Under the catalysis of GPX4, lipid peroxides are reduced to phospholipid molecules, thereby preventing excessive accumulation of lipid peroxides. FTH1, as an important protein for storing and releasing iron, can protect cells from the toxic effects of free iron ions (Qiu et al., 2023; Tang et al., 2023). Undoubtedly, the expression of SLC7A11, GSH, and GPX4 proteins could slow down the process of ferroptosis, and the expression of these factors were inhibited in our study (Figures 3A and 5). We inferred that mitochondrial damage acted as a trigger to advance redox imbalance in the body, accelerated the process of lipid peroxidation, and triggered ferroptosis under MPs exposure.

CONCLUSIONS

In summary, our study showed that MPs exposure can damage the mitochondrial structure, give rise to the disorder of mitochondrial function, trigger apoptosis of the mitochondrial pathway, and stimulate redox imbalance-induced ferroptosis, thus inducing damage to spleen tissue. This work centered on mitochondrial damage to explore the specific mechanism of MPs' damage to the spleen of chicken, providing a supplement to the laboratory toxicology data of MPs-chicken.

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DISCLOSURES

The authors declare no conflicts of interest.

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