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RESEARCH ARTICLE

Evaluating the Hepatotoxicity of Polyvinyl Chloride Microplastics in Ducks: Oxidative and Fibrotic Outcomes

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that is common world-wide and studied extensively in mammals. Nevertheless, the pathways of NAFLD associated liver damage in the digestive tracts of birds remain unclear. In this study, 7-day-old female Muscovy ducks of the same batch were split into three groups and were fed pure water, 1mg/L polyvinyl chloride microplastics (PVC-MPs) or 10mg/L PVC-MPs, respectively, for 2 months. This study aimed to verify whether oxidative stress originated from PVC-MPs resulted in NAFLD in duck liver, thus triggering apoptosis in hepatocytes. The results of the study proved that the accumulation of PVC-MPs in liver had adverse effects on the morphological structure and functional performance of liver cells. This finding was supported by a decrease of liver organ coefficient and pathological injury to liver cells, as well as ultrastructural injury. Oxidative stress injury in the liver of female breeding duck induced the deficiency of PCK1 and activated PI3K/AKT pathway, which resulted in fatty deposition and fibrosis in the liver and led to hepatocyte apoptosis. In conclusion, PVC-MPs induced hepatic oxidative damage, hepatic lipid deposition and hepatic tissue fibrosis, and hence hepatic apoptosis. Our research provides new perspectives on the association of PVC-MPs with liver toxicity in female duck that contains hepatic dysfunction, mainly NAFLD.

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INTRODUCTION

Plastics are overwhelmingly used that raise environmental concerns. Secondary microplastics (MPs) generated by MP polymer degradation exist ubiquitously and persist in ecosystems through atmospheric and hydrological processes (Sun *et al.*, 2022). The MPs pollution of Chinese aquatic systems is serious, and in collected samples, 89.8% of the species surveyed (230 of 256) shows bio-accumulation (Xu *et al.*, 2023). Birds are extremely sensitive to MPs exposure. *Anatidae* waterfowl including some of the most economically important duck stocks with global annual production, are simultaneously exposed through consumption from aquatic habitats and the atmosphere (Reynolds and Ryan, 2018). MPs found in lungs and in gastrointestinal tract cause mechanical tissue damage (intestinal perforation), metabolic tissue damage (pseudo satiety) and systemic damage (inflammatory response, immunodeficiencies) (Wright *et al.*, 2013). Although there is the recognition of hepatic MPs presence in marine animals, no studies report PVC-MPs bioaccumulation in duck liver which is an important research gap as the organ is metabolic and has a high economic value (Fossi *et al.*, 2018). This gap must be urgently investigated to study the hepatotoxic impact and food safety in poultry products on human consumption (Chen, *et al.*, 2021; Liu, *et al.*, 2023).

The liver is a major bioaccumulation target of MPS and has been the subject of studies on the effects of its intake. Various animal models have demonstrated the hepatotoxicity of MPs, primarily in aquatic animals (Shen, *et al.*, 2022; Wang, *et al.*, 2022a). Oral administration of

0.3 and 3.0μg/mg of polystyrene microplastics (PS-MPs) with particle sizes (2 and 200μm) for 28 days resulted in significant intestinal flora disorders and disturbances of the enterohepatic axis in marine medaka (Feng, *et al.*, 2021). However, mice exposed to high concentrations of PS-MPs for 8 weeks showed significant changes in genes related to lipid metabolism pathways, according to transcriptomics results. Additionally, lipidomic results revealed alterations in hepatic free fatty acids (FFAs) and triacylglycerols (TAGs) (Wang, *et al.*, 2022b). In addition, studies have shown that PVC-MPs are uniquely carcinogenic to the liver (Zarus, *et al.*, 2023). However, unfortunately, no literature reports exist on the mechanism of liver injury in ducks caused by PVC-MPs.

As obesity, diabetes, hyperlipidemia, and cardiovascular disease are becoming more common problem globally and NAFLD, has emerged as an increasing chronic liver condition. Mice exposed to PVC-MPs resulted in altered gut microbiota and liver damage (Chen *et al.*, 2022). The study also found that hepatic steatosis was further enhanced in HFD mice and pa-induced hepatocyte cell lines exposed to PVC-MPs (Jin *et al.*, 2021). A recent research found that PVC had the highest association with hepatotoxicity among all microplastics (Jin *et al.*, 2021). Therefore, MPs-induced metabolic alterations may contribute to the development of NAFLD (Auguet *et al.*, 2022). However, there is a scarcity of evidence regarding the potential role of PVC-MPs accumulation in NAFLD in avian species.

The objective of this study was to investigate the effects and mechanisms of action of PVC-MPs on duck liver NAFLD. The study involved characterizing and quantifying hepatic microplastics in farmed breeder ducks, assessing levels of oxidative stress injury, and screening key genes that could regulate NAFLD transcriptomics to clarify the effects and mechanisms of action of PVC-MPs exposure on hepatic NAFLD in ducks.

MATERIALS AND METHODS

Chemicals and reagents: Table 1 listed the routine reagents used in this study while Table 2 listed information about the antibodies used in this study. Analyst-grade chemicals and reagents were bought locally.

Animal experiment: Female Muscovy ducks (n=24) were reared under constant temperature conditions (25°C ± 2°C, 72% humidity) and randomized into three groups: (1) control (basal diet), (2) low-dose (1mg/L PVC-MPs in drinking water), and (3) high-dose (10mg/L PVC-MPs), reflecting environmental concentrations in China's Yangtze River Basin (Yin *et al.*, 2023). Each diet used for experiment followed an NRC nutritional standard (Shakouri and Malekzadeh, 2016). Animal care protocols followed those notified by Yangzhou University (SYXK[Su]2021-0027).

Samples collection: After 60 days, ducks were killed humanely by intramuscular injection of xylazine and ketamine. Blood was collected from the median wing vein of the ducks to obtain plasma. The tissues were stored at -80°C for later examinations or were fixed with 2.5% glutaraldehyde or 4% paraformaldehyde for histological analysis.

Table 1: Drugs and kits used in present study

Name	Catalog No.	Company
PVC-MPs	202908	Zhichuan
MDA	A003-1-2	Jiancheng
SOD	A007-1-1	Jiancheng
CAT	A001-3-2	Jiancheng
GSH	A006-2-1	Jiancheng
TG	A110-1-1	Jiancheng
T-CHO	A111-1-1	Jiancheng
HDL-C	A112-1-1	Jiancheng
LDL-C	A113-1-1	Jiancheng
ALT/AST	Z002-1-1	Jiancheng
H&E Stain Kit	G1120	Solarbio
Masson Stain Kit	G1340	Solarbio
TUNEL Assay Kit	C1086	Beyotime
BCA Kit	P0009	Beyotime
Oil Red O Stain Kit	C0158M	Beyotime
Sirius Red Stain Kit	G1472	Solarbio
BODIPY 493/503	GC42959	GLPBIO

Table 2: Antibodies used in the present study.

Antibody	Catalog number	Company
Bax	T40051	Abmart
Bcl-2	T40056	Abmart
Caspase-3	T40044	Abmart
Caspase-9	T40046	Abmart
AKT	60203-2-Ig	Proteintech
p-AKT	66444-1-Ig	Proteintech
PI3K	60225-1-Ig	Proteintech
α-SMA	80008-1-RR	Proteintech
TGF-β1	21898-1-AP	Proteintech
COL4A1	19674-0-AP	Proteintech
PCK1	sc-271029	Santa Cruz
GAPDH	66009-1-Ig	Proteintech
Goat anti-rabbit	7074	Cell Signaling Technology
Horse anti-mouse	7076	Cell Signaling Technology

Cell culture and treatment: Twenty-one-day-old duck embryos were purchased from the Gaoyou Duck Breeding Base (Yangzhou, China). Primary duck hepatocytes (PDH) were isolated using the liver perfusion technique. Cell purity, verified through periodic acid–Schiff (PAS) glycogen staining, exceeded 95%. The hepatocytes were cultured in Williams' E-medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin.

Bio-chemical analysis: Liver samples were homogenized to prepare a 10% (w/v) tissue homogenate. Enzymatic activities of alanine transaminase (ALT), malondialdehyde (MDA), catalase (CAT), glutathione (GSH), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) were determined using commercial assay kits following standardized protocols.

Trace Element Analysis: Lyophilized samples were finely ground and subjected to microwave digestion using 5mL nitric acid and 1mL hydrogen peroxide (H_2O_2). The concentrations of trace elements were quantified by flame atomic absorption spectrometry (FAAS; PerkinElmer Optima 3700DV).

Determination of PVC-MPs in liver by immunofluorescence (IF): The liver fixed in 4% paraformaldehyde was dehydrated with 20% sucrose before sectioning. Fluorescent MPs in liver were visualized by microscope (TCS SP8 STED, Leica).

Histological Analysis: Fixed liver tissue was dehydrated using an ethanol gradient, paraffin-embedded, and sectioned. Hematoxylin and eosin (H&E) staining was performed according to the standard instructions. Histomorphology evaluation was performed using a Leica DMI3000B inverted microscope with bright-field illumination.

Transmission Electron Microscopy (TEM): The livers fixed in 2.5% glutaraldehyde at room temperature were placed at 4°C for 12 hours. The samples underwent staining, ultra-thin sectioning, entrapment, dehydration, fixing in osmic acid, and transmission electron microscopy observation.

TUNEL Staining: The TUNEL assay was utilized to detect apoptosis in damaged livers using formaldehyde-fixed paraffin-embedded sections, as per the manufacturer's instructions.

Immunohistochemistry (IHC): Liver sections were dewaxed in xylene, dehydrated using an ethanol gradient, and blocked for endogenous peroxidase activity with 3% H_2O_2. Liver sections were incubated with primary antibody at 4°C overnight, followed by HRP-conjugated secondary antibody. DAB chromogen visualized signals. Results were observed by microscope (DMI3000B, Leica, Germany).

Identifying types of MPs by PY-GC/MS : MPs content in duck liver was analyzed by Py-GC/MS using a Frontier Lab PY-3030D pyrolyzer with platinum filament and Shimadzu GCMS-QP2020. Powdered samples were acid-digested (HNO_3, 2h), neutralized with deionized water, and solvent-evaporated prior to analysis. Quantification employed a standard calibration curve derived from MPs reference materials, with peak area integration for MPs determination. All analyses were conducted by Shanghai Weipu Testing Technology Group.

RNA sequencing (RNA-seq) analysis: RNA-seq libraries were prepared using the ABclonal mRNA-seq Library Preparation kit (ABclonal, China). Functional enrichment analyses of genes were carried out using the ClusterProfiler package. Enriched pathways and gene ontology (GO) terms were further examined through Kyoto Encyclopedia of Genes and Genomes (KEGG) database using RStudio and the DAVID bioinformatics tool.

Western Bolting: Liver was homogenized in a protease inhibitor-containing buffer, pelleted and sonicated. Supernatants were quantified for protein using BCA kit. Proteins were loaded on SDS-PAGE and transferred on PVDF membranes. Blots were blocked with 5% milk, and incubated overnight with primary and secondary antibody, followed by wash with TBST. Chemiluminescent signals were captured and counted with ImageJ (NIH), and GAPDH normalized. All steps are triplicates of biological replicates.

Statistical analysis: Data analysis was performed by GraphPad Prism 9 software. The results from three independent experiments were evaluated by parametric tests. Results are shown as the mean ± SD and regarded as statistically significant when $P<0.05$.

RESULTS

Characterization and accumulation of PVC-MPs in liver: For MPs accumulation in the hepatic tissue of 120-day-old Muscovy ducks, Py-GC-MS analysis showed absent PS but detectable amounts of PP (11.21mg/kg), PA66 (28.47mg/kg), PE (64.29mg/kg) and PVC (76.26mg/kg) (Fig. 1A), so PVC-MPs was chosen for further experiments because of the highest bioavailability.

FTIR and SEM confirmed that PVC-MPs were characteristic in their absorption peak, uniform spherical form (5 μm in size), and smooth surface (Fig. 1B & C). IF demonstrated penetration of PVC-MPs into periductal tissue of liver (Fig. 1D). The cryosections also confirmed dose-dependent liver concentration of PVC-MPs (Fig. 1E).

Effect of PVC-MPs on morphology and functional activity of liver: Liver tissue showed no apparent morphological changes, suggesting negligible structural changes of PVC-MPs (Fig. 2B). However, animals exposed to PVC-MPs had reduced body weight, liver weight and hepatosomatic index than control group (Fig. 2C). Serum ALT of MPs exposure ducks was significantly elevated when compared with control animals, which suggested liver damage and physiological disturbance (Fig. 2D). For all exposure doses, dose-dependent histopathological changes were observed: 10mg/L PVC-MPs caused more serious hepatic cord disorganization and steatosis than 1mg/L PVC-MPs (Fig. 2E).

TEM of hepatocytes showed preserved nuclear morphology and normal morphologies of mitochondria in control group. TEM of hepatocytes showed nuclear membranes constriction and widened pore complex in 10mg/L PVC-MP as compared to 1mg/L PVC-MP. In high dose, mitochondrial inner membrane not only became fragmented but also showed even more pronounced intramitochondrial vacuolation (Fig. 2F).

Effects of PVC-MPs on oxidative stress in duck liver: PVC-MP exposure altered redox homeostasis in liver, inducing a dose-dependent reduction of T-AOC, GSH and SOD and increase of MDA and CAT levels in liver, compared to the control group, and the dose efficacy response for the high doses were greater than that for 1mg/L PVC-MPs (Fig. 3A-E).

FAAS analysis showed substantial hepatic accumulation of Cu and Mn in 10mg/L PVC-MPs versus control and 1mg/L PVC-MPs groups (Fig. 3F&I). It is noteworthy that 1mg/L PVC-MPs uniquely elevated iron levels versus control group, compared with the higher doses (Fig. 3G). Lastly, the concentrations of zinc were similar across all the different groups (Fig. 3H).

Transcriptome analysis suggests that exposure to PVC-MPs affects the function of duck liver by regulating the PCK1-PI3K/AKT signaling pathway: RNA sequencing analysis showed obvious downregulation of the PCK1 (Fig. 4A). Pathway enrichment analysis showed PPAR and PI3K/AKT signaling as major modulated pathways (Fig. 4B), and GSEA analysis shows the regulation of

PI3K/AKT pathway was dysregulated (Fig. 4C), these all suggest that the PVC-MPs may promote the progression of NAFLD through PCK1-PI3K/AKT signaling-PPAR axis. Network analysis and Western blot validation showed coordinated suppression of PCK1 with coordinated activation of the PI3K/AKT-PPAR pathway (Fig. 4D-F).

Effect of PVC-MPs on lipid accumulation in the liver tissue: Histopathological examination of the effect on hepatic concentrations showed dose-dependent accumulation of lipids, with a striking lipid droplet vacuolization in 10mg/L PVC-MPs, compared to 1mg/L PVC-MPs and showed such dose-dependent ultrastructural lipid deposition by TEM (Fig. 5A). At the same time, hepatic triglyceride (TG) and total cholesterol (T-CHO) increased by 38% and 27% in both high doses, accompanied by changes in hepatic tissue HDL-LDL (Fig. 5B).

However, the same results were produced by PDH. BODIPY staining found a high fold-increase in the quantity of lipid droplets in the 250μg/ml group compared to that of the 31.25μg/ml group (Fig. 5C), similar results were found using Red Oil staining (Fig. 5E). The kit represented a clear increase of TG, T-CHO, HDL-C, LDL-C levels in the 250μg/ml group compared with 31.25μg/ml group (Fig. 5D).

Effect of PVC-MPs on collagen fibers in the liver tissue: Histopathologic Sirius Red and Masson staining results suggested severe collagen accumulation in the whole liver of PVC-MPs-treated animals when compared with control group, with collagen increase showing obvious doses-dependent level in 1mg/L and 10mg/L PVC-MPs groups (Fig. 6A). Histopathological IHC staining showed increased expression of α-SMA and TGF-β1 levels in 10mg/L PVC-MPs group (Fig. 6A). Western blotting further confirmed upregulation of fibrotic markers (α-

(A)

Tissues	Numbers	MPs types&Abundance (mg/kg)										
		PS	PE	PP	PMMA	PVC	PC	PET	PA6	PA66	PLA	PBAT
Liver	1	N.D.	120.5097	8.8655	N.D.	99.6057	N.D.	N.D.	N.D.	22.7006	N.D.	N.D.
	2	N.D.	50.3590	3.9832	N.D.	112.0633	N.D.	14.7739	N.D.	42.9347	N.D.	N.D.
	3	N.D.	7.7297	10.0470	N.D.	38.1423	N.D.	9.1841	N.D.	18.4458	N.D.	N.D.
	4	N.D.	133.1786	24.5952	N.D.	54.5978	N.D.	N.D.	N.D.	17.7684	N.D.	N.D.
	5	N.D.	9.6625	8.5291	N.D.	76.8705	N.D.	25.8791	N.D.	40.4931	N.D.	N.D.
	Mean	**N.D.**	**64.2947**	**11.2091**	**N.D.**	**76.2560**	**N.D.**	**16.6124**	**N.D.**	**28.4685**	**N.D.**	**N.D.**

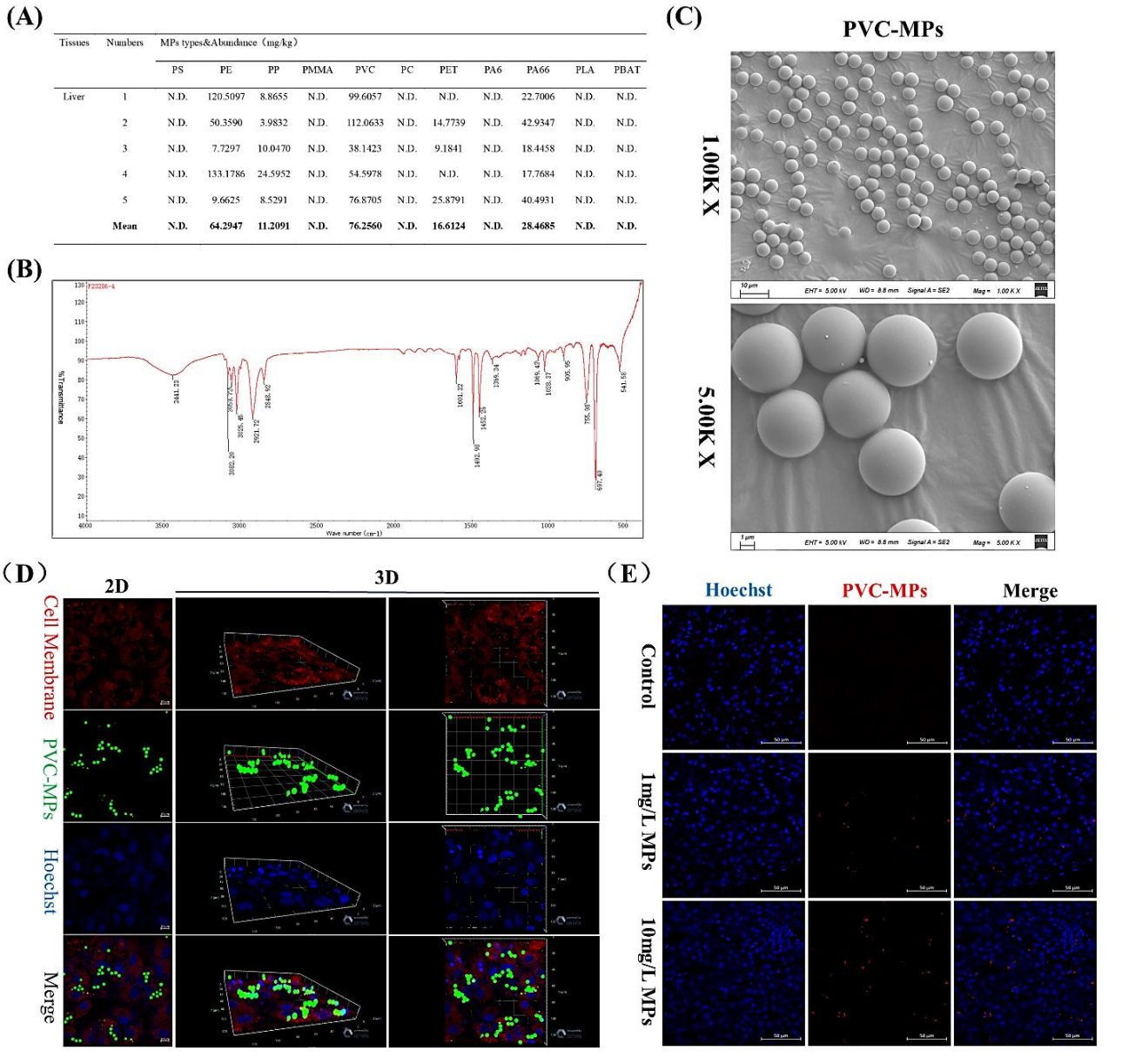

Fig. 1: Microplastic residue can be detected in duck liver. (A) Microplastic types and abundance of duck liver by Py-GC-MS. (B&C) Characteristic observation of PVC-MPs through FTIR and SEM. (D) PDH were treated with different concentrations of PVC-MPS for 12 hours. Accumulation of PVC MPs in PDH by laser confocal microscopy. Red (CY3) indicates the cell membrane. Green (GFP) represents FPS-MPs. (E) Representative image of liver section from duck exposed for 5μm FPS-MPs for 60 days. Red (RFP) indicates FPS-MPs. Blue (Hoechst) represents the cell nucleus.

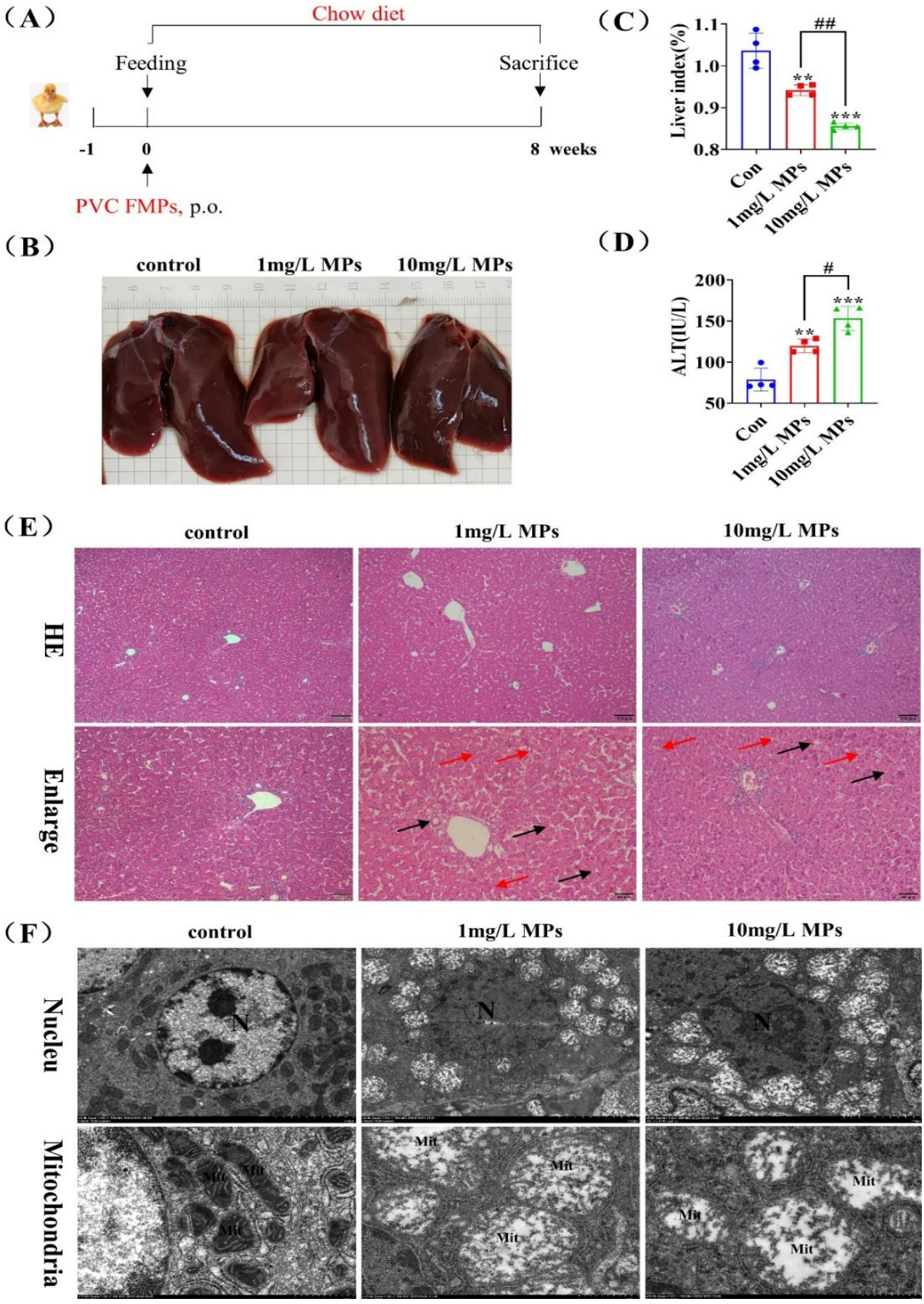

Fig. 2: Effect of PVC-MPs on the morphology and functional activity of duck liver. Ducks were fed different concentrations of PVC-MPs for 60 days. (A) Animal experiment flowchart. (B) Effects of different concentrations of 5μm PVC-MPs exposure for 60 days on duck liver index. (C) Morphological observation of the liver. (D) Serum ALT levels of each group. (E) H&E staining of each group. Red arrow indicates inflammatory cell infiltration. Black arrow indicates disruption of hepatic cords. (F) TEM observation nucleus and mitochondria of each group. Results are shown as the mean±SD. Independent sample t-tests were performed the significance of differences between groups. **P<0.01; ***P<0.001; # P<0.05; ##P<0.01. n=4.

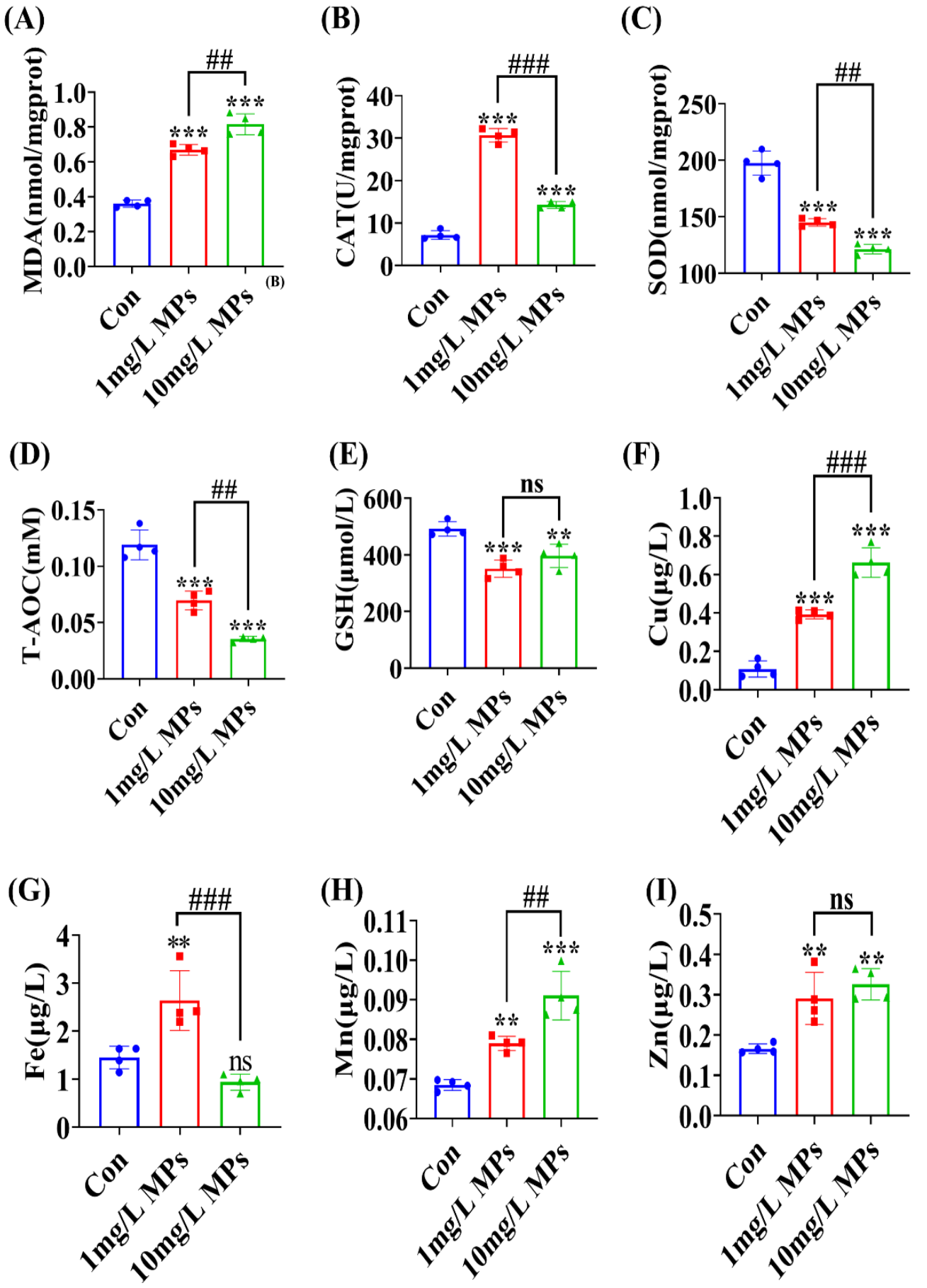

Fig. 3: Microplastics promote oxidative stress in the liver. Ducks were fed different concentrations of PVC-MPs for 60 days. (A-E) Oxidative enzymes MDA, and anti-oxidative enzymes CAT, SOD, T-AOC and GSH levels of each group. (F-I) Fe, Mn, Zn, and Cu contents of each group. Results are shown as the mean±SD. Independent sample *t*-tests were performed the significance of differences between groups. **P<0.01; ***P<0.001; ##P<0.01; ###P<0.001; ns P>0.05. n=4.

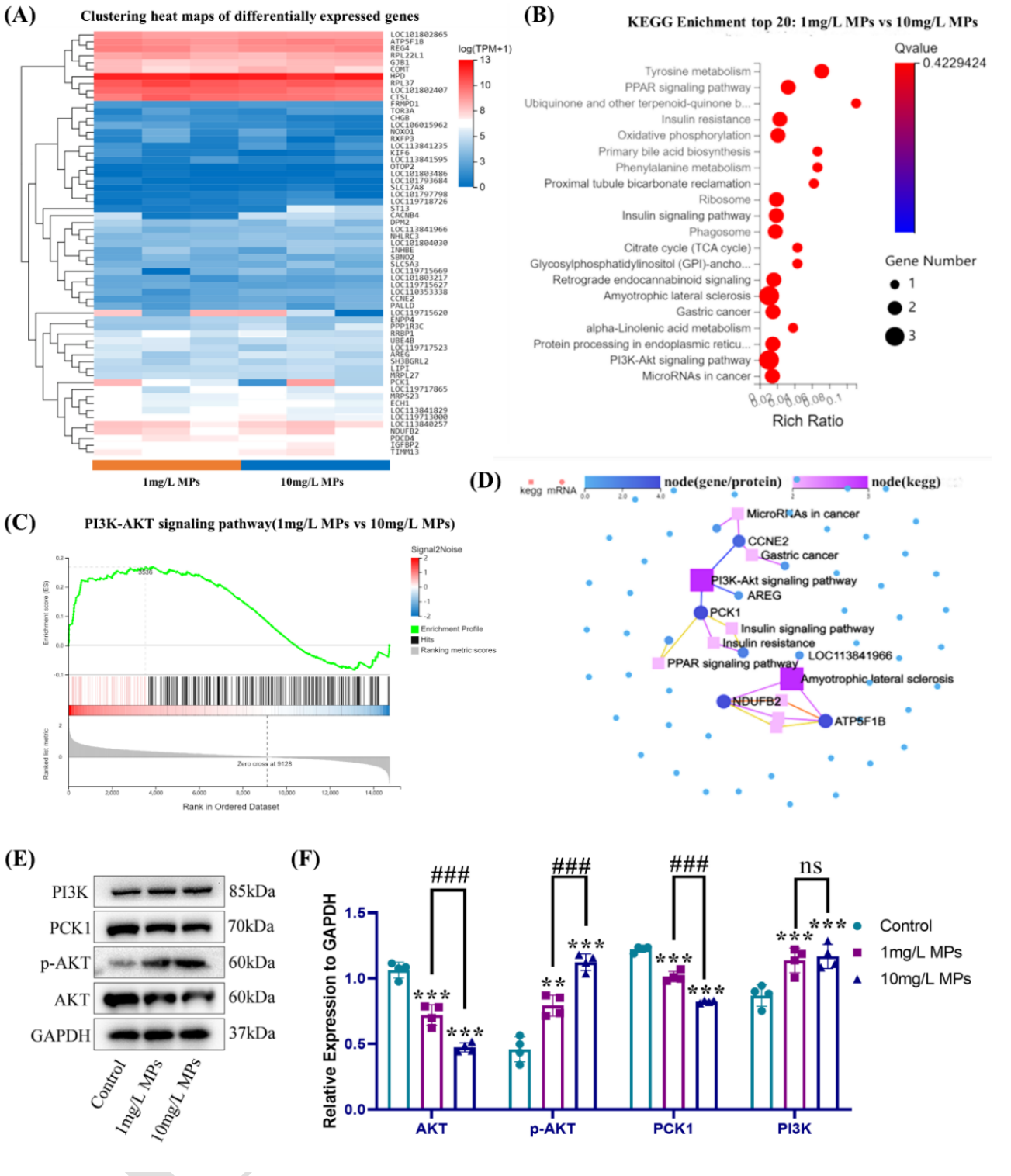

Fig. 04: Transcriptome analysis reveals that PVC-MP exposure affected duck liver function by regulating genes involved in glucolipid metabolism. Ducks were fed different concentrations of PVC-MP for 60 days. (A) Heat map of transcriptome expression of each sample. (B) The significantly enriched KEGG pathways of DEGs. (C) GSEA analysis of RNA-seq data from PVC-MPs exposure duck liver. (D) Transcriptomics regulatory network map of DEGs. (E-F) Protein expression levels of PCK1, PI3K, AKT, p-AKT, and GSK-3β of each group. Results are shown as the mean±SD. Independent sample t-tests were performed the significance of differences between groups. **P<0.01; ***P<0.001; ###P<0.001; ns p>0.05. n = 4.

SMA, TGF-β1, COL4A1) in PVC-MPs-treated livers, with more pronounced changes at higher concentrations (Fig. 6B-E). These findings collectively indicate that PVC-MPs may induce hepatic fibrosis in a dose-responsive manner.

Effect of PVC-MPs on apoptosis in the liver tissue: TUNEL staining revealed dose-dependent apoptosis elevation in PVC-MPs-exposed liver tissues, with higher positivity in the 10mg/L group versus 1mg/L and controls (Fig. 7A). Immunohistochemistry confirmed upregulated Caspase-3 and Caspase-9 expression in treated groups, intensifying at 10mg/L (Fig. 7A). Western blotting also showed the dysregulation of apoptotic markers (Bax, Bcl-2) of the PVC-MPs induced apoptosis, with relatively more obvious changes at 10mg/L exposure (Fig. 7B-E).

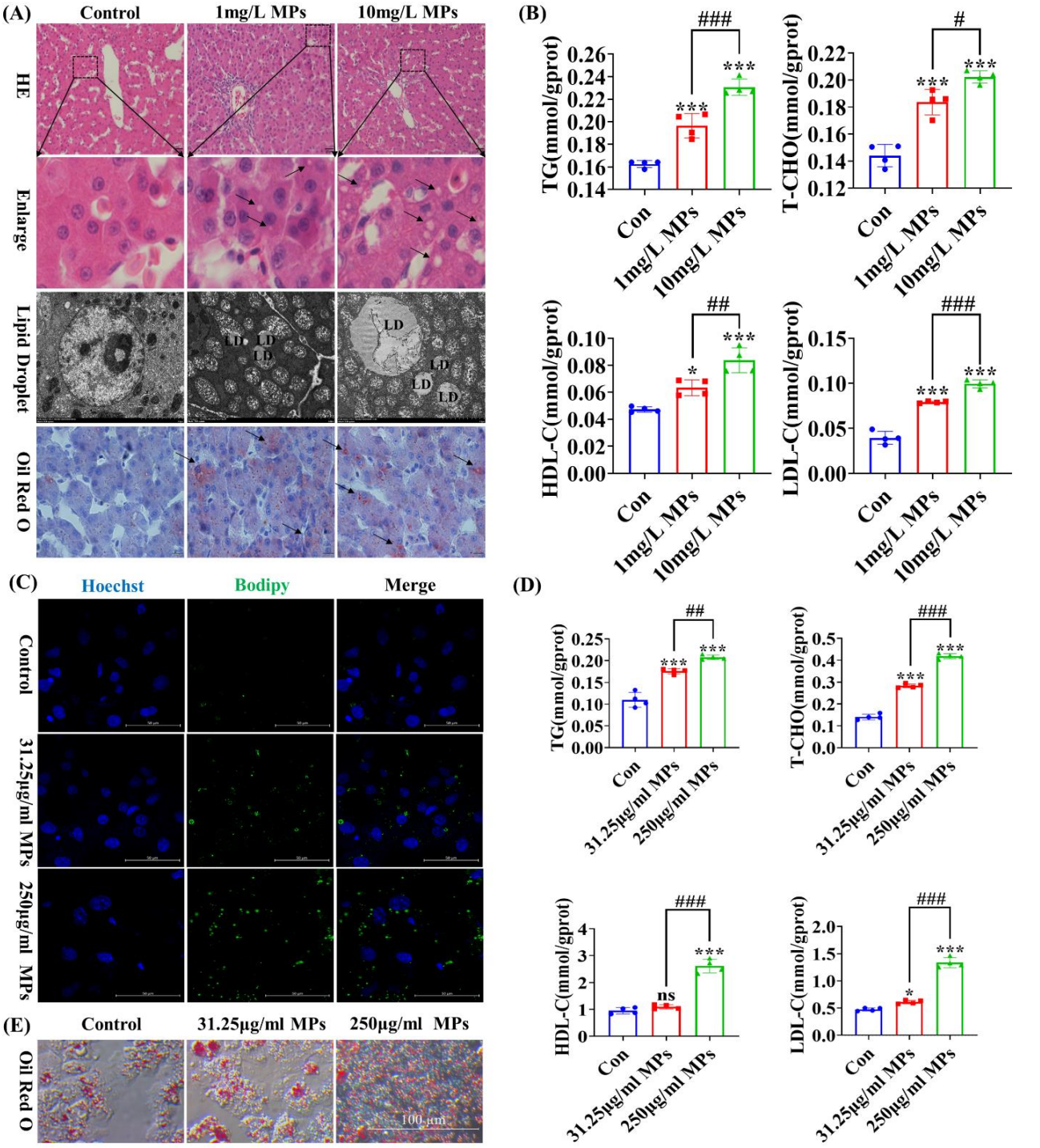

Fig. 5: Microplastics promote lipid accumulation in the liver. Ducks were fed different concentrations of PVC-MP for 60 days. (A) Lipid deposition levels of each group. Black arrow indicates lipid droplets. (B) TG, T-CHO, HDL-C, and LDL-C levels of each group. PDH were treated with different concentrations of PVC-MPS for 12hours. (C&E) Lipid deposition levels in PDH of each group. Green (GFP) represents lipid droplets. Blue (Hoechst) represents the cell nucleus. (D) TG, T-CHO, HDL-C, and LDL-C levels in PDH of each group. Results are shown as the mean±SD. Independent sample *t*-tests were performed the significance of differences between groups. *P<0.05; ***P<0.001; #P<0.05; ##P<0.01; ###P<0.001; ns P>0.05. n=4.

DISCUSSION

Although MPs are emerging environmental threats with well-known impacts on mammals, studies of avian toxicity are scarce and mainly based on PS-MPs (Zhang, *et al.*, 2022; Lu *et al.*, 2023). This pioneering study demonstrates that PVC-MPs are hepatotoxic to female ducks and considers, for the first time in avian toxicology, chronic exposure of MPs to cause hepatic dysfunction and even NAFLD development.

Evidence is growing on MPs accumulation in livestock systems including manure, feed and breeder duck muscle (Chen *et al.*, 2023). Our study is also the first report of MPs residue in farmed breeder ducks in China with predominance of PVC and PE MPs residues. Possible sources could be PE bag lines for feed bottles and PVC water pipes but ducks had an indoor livable environment and were not externally supplied with plastics. More interestingly, Py-GC-MS detection capabilities overcome size restrictions, facilitating the characterization of MPs in

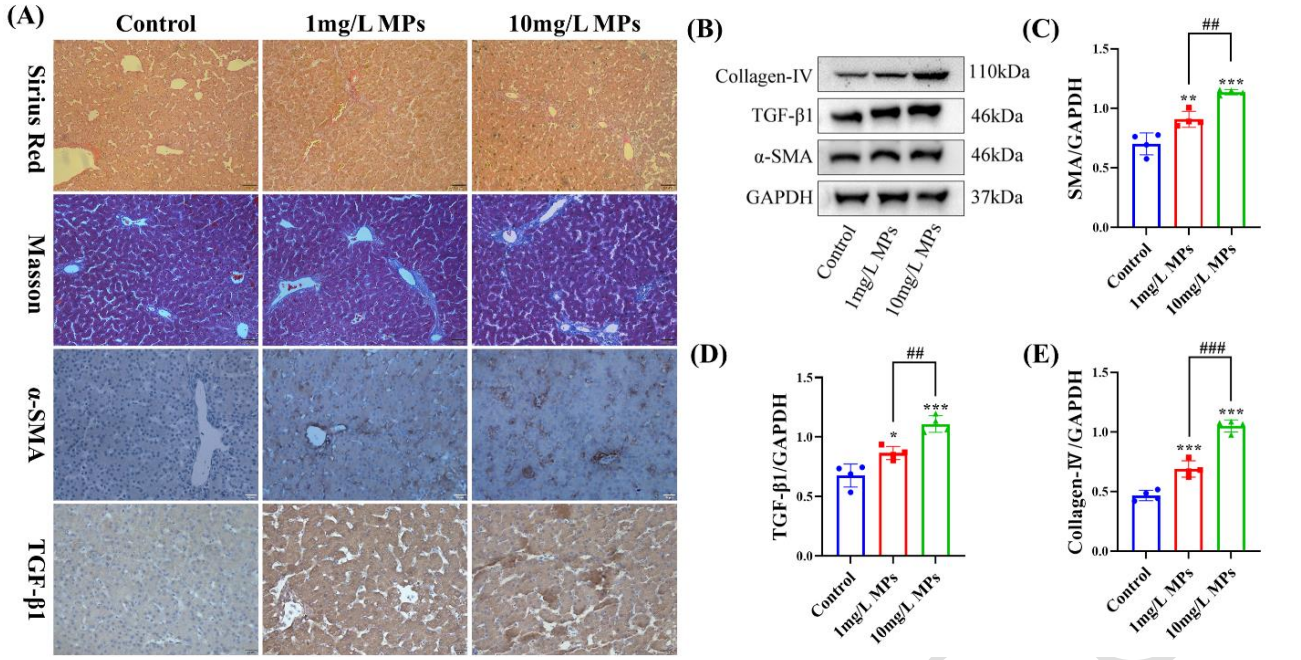

Fig. 6: Microplastics promote liver fibrosis. Ducks were fed different concentrations of PVC-MP for 60 days. (A) Collagen fibers levels of each group. Positive areas and intensities of α-SMA and TGF-β1 in each group. (B-E) Protein expression levels of α-SMA, TGF-β1, and COL4A1 of each group. Results are shown as the mean±SD. Independent sample t-tests were performed the significance of differences between groups. *P<0.05; **P<0.01; ***P<0.001; ##P<0.01; ###P<0.001. n=4.

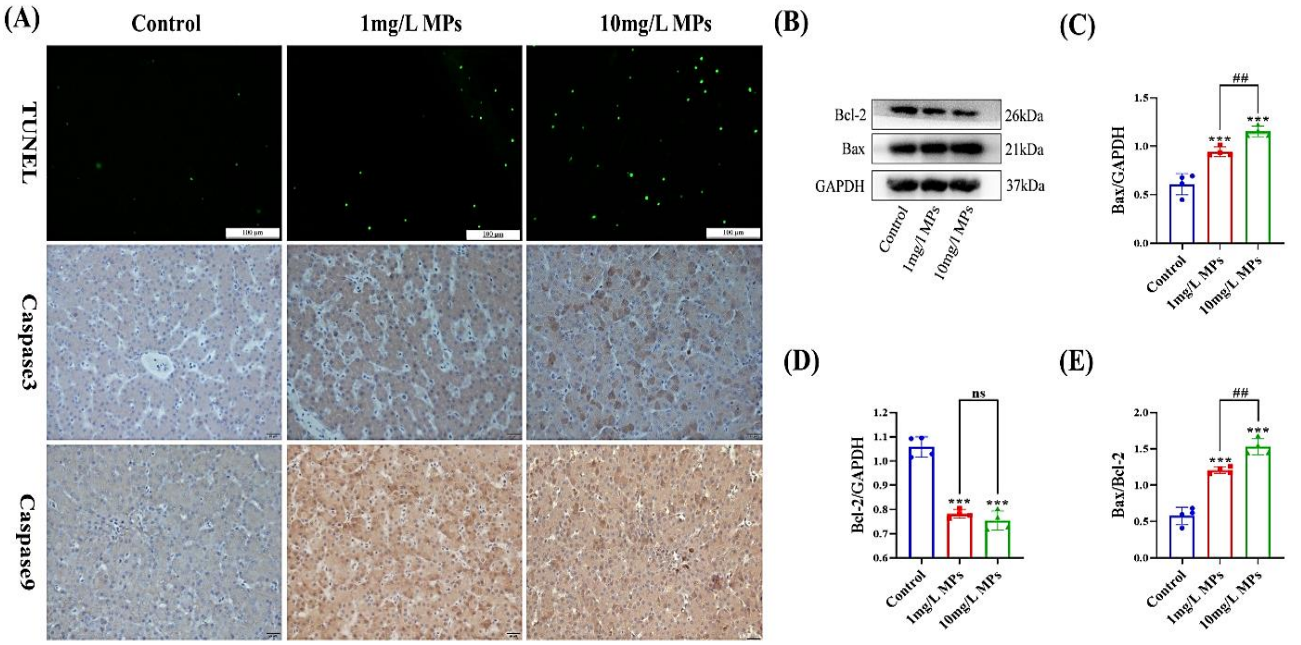

Fig. 7: Microplastics promote liver apoptosis. Ducks were fed different concentrations of PVC-MP for 60 days. (A) Number of apoptotic cells of each group. Positive areas and intensities of Caspase-3 and Caspase-9 in each group. (B-E) Protein expression levels of Bax and Bcl-2 of each group. Results are shown as the mean ± SD. Independent sample t-tests were performed the significance of differences between groups. ***P<0.001; ##P<0.01; ns P>0.05. n=4.

a biological matrix (XiaoZhi, 2021;Marfella *et al.*, 2024). To reduce potential contamination, we ask feed manufacturers to minimize MPs shedding from friction by feeding bags and to switch the PVC water system to a healthier system.

These results led us to further animal experiments using 5μm PVC-MPs. We observed typical dose-dependent accumulation of MPs in duck liver tissues and liver cells, which is consistent with previous reports of MPs bioaccumulation in aquatic animals (Yu *et al.*, 2023). Histopathology showed pronounced hepatic vacuolization, nuclear solidity formation and mitochondrial ultrastructural damage, exhibits oxidative dysfunction. These changes are consistent with changes on lipid metabolism and initiation of apoptosis (Baybutt *et al.*, 2002). PVC-MPs induced the increase in level of hepatic MDA in ducks and decreased the activities of hepatic SOD, GSH and T-AOC, implying oxidative stress. Paradoxical effect of CAT was up-regulation, which showed a certain magnitude of counteracting antioxidant effect (Shengchen *et al.*, 2021). As ROS-driven oxidative stress is pivotal in

steatohepatitis, our findings establish PVC-MPs as inducers of redox imbalance and metabolic injury in avian models (Mann *et al.*, 2017).

Our transcriptome results revealed great differences in the expression of a large number of genes involved in glycolipid metabolism after PVC-MPs exposure. Analysis of liver physiological functions also showed that PVC-MPs exposure may cause hepatotoxicity and exacerbate accumulation of lipids in the liver. Hepatic lipid metabolism and glucose metabolism are interacting relationship (Bechmann *et al.*, 2012). PCK1 is the first regulatory enzyme in the process of gluconeogenesis that converts cytoplasm oxaloacetate into phosphoenolpyruvate (Tuo *et al.*, 2019). In our study results, the PCK1 gene was significantly downregulated and particularly enriched in the PI3K/ATK pathway. A recent study showed that loss of the gluconeogenic enzyme PCK1 from male mice triggered NAFLD development and function by activating PI3K/AKT pathway (Ye *et al.*, 2023), indicating that the PI3K/ATK pathway contributes to the effect of PVC-MPs on NAFLD of ducks.

Our data revealed that PVC-MPs caused dose-dependent liver steatosis and fibrosis, central hallmarks of NAFLD progression to NASH (Ye *et al.*, 2023). High dose of PVC-MPs enhanced the lipids accumulation both *in vivo* and *in vitro*, as reported for fibrosis severity at a certain size for PS-MPs-fed models (Wang *et al.*, 2023). Notably, PVC-MPs had stronger fibrogenic effects than PS-MPs, possibly accounting for its prominent NAFLD aggravation in ducks. Microscopy has also revealed elevated hepatocyte necrosis and apoptotic damage to correlate with oxidative stress-mediated damage. The same apoptotic responses to PS-MPs shown in duck hepatic organs for avian organs indicate the oxidative injury as a common route for microplastic toxicities regardless of MPs type (Hou *et al.*, 2022; Li *et al.*, 2023). Therefore, these data position PVC-MPs as high-risk pollutants triggering metabolic and structural pathological liver effects in birds.

Conclusions: The present study shows the occurrence of liver toxicity of PVC-MPs in duck livers, which may lead to progresses of NAFLD in duck livers through exposure of female breeding ducks. PVC-MPs exposure decreased their hepatic organ coefficient, increased their serum ALT level and causing liver nuclear and mitochondrial ultrastructure damage. Mechanism-wise, PVC-MPs exposure destroyed the hepatic redox homeostasis, leading to oxidizing stress-induced NAFLD. Deficiency of hepatic PCK1 in ducks also activated the PI3K/AKT pathway, which caused lipid deposition and fibrosis worsened. Transcriptomics revealed the target genes underlying the hepatic metabolic syndrome induced by PVC-MPs. Thus, our study revealed PVC-MPs as an important pollutant inducing hepatic toxicity in birds, which may attribute to oxidative damage, fibrotic pathways, and havoc caused by disturbances in glycolipid metabolism. However, elucidating precise molecular mechanisms requires complementary *in vitro* models to validate clinical relevance.

Data availability: The raw data for the Western blotting are presented in Supplementary Fig 1. Additional relevant data are available from the corresponding author.

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Declarations

Consent for publication: Not applicable

Declaration of competing interest: The authors declare that there are no conflicts of interest.

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