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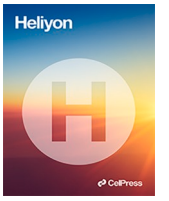

The impact of dioctyl phthalate exposure on multiple organ systems and gut microbiota in mice

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ABSTRACT

Dioctyl phthalate, commonly known as bis(2-ethylhexyl) phthalate (DEHP), is a widely used plasticizer in various industries and has been shown to directly or indirectly impact human health. However, there is a lack of comprehensive studies evaluating the potential health risks associated with DEHP accumulation in different organs across various age groups. This study aimed to assess the effects of low (50 mg/kg·bw) and high (500 mg/kg·bw) doses of DEHP on five different organs in mice at young (4-week-old) and aged (76-week-old) life stages. Our findings revealed that both low and high doses of DEHP exposure led to significant dose-dependent inflammation in the liver, spleen, and kidney. Furthermore, regardless of age, DEHP exposure resulted in elevated activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the liver, as well as increased levels of creatinine (Cr) and urea in the kidney. Moreover, analysis of the fecal microbiota using 16S rRNA sequencing demonstrated that DEHP exposure disrupted the homeostasis of the gut microbiota, characterized by an increased abundance of pathogenic bacteria such as *Desulfovibrio* and *Muribaculum*, and a decreased abundance of beneficial bacteria like *Lactobacillus*. This study provides compelling evidence that DEHP at different concentrations can induce damage to multiple organs and disrupt gut microbiota composition. These findings lay the groundwork for further investigations into DEHP toxicity in various human organs, contributing to a better understanding of the potential health risks associated with DEHP exposure.

1. Introduction

Dioctyl phthalate (DEHP) is a widely used lipophilic compound in various industries [1]. Due to its tendency to leach and enter environmental cycles, phthalates can easily impact human health [2]. DEHP has been detected in soil samples from multiple cities in

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China, leading to traces of this compound in food and drinking water [2]. Human exposure to DEHP can occur through various routes, including oral ingestion, inhalation, and skin contact. Additionally, DEHP in soil can enter plants and subsequently enter the human or animal food chain, potentially leading to carcinogenic and mutagenic effects [1,3,4]. Recent studies have detected DEHP in various human tissues and fluids, such as the liver, blood, umbilical cord blood, breast milk, placenta, and amniotic fluid during early pregnancy [5,6]. Long-term exposure to plastic products, including DEHP, has been linked to sexual precocious puberty in children and reproductive dysfunction [7]. In adults, DEHP exposure has been associated with liver inflammatory responses, hepatocyte apoptosis, testicular cancer, ovarian cancer, lymphatic epithelial damage, intestinal function disorders, alterations in gut microbiota, and reproductive defects [8–15]. Notably, significant morphological differences have been observed in the heart, kidney, and lungs of old and young rats, providing an experimental basis for studying chronic diseases and understanding organ dysfunction in the elderly [16]. However, limited attention has been given to the potential damage caused by different doses of DEHP exposure to multiple organs in animals at different life stages.

The gut microbiota plays a crucial role as the first line of defense against ingested environmental xenobiotics [17]. Emerging evidence has highlighted the association between gut microbiota and various physiological functions that contribute to maintaining overall health [18,19]. Importantly, exposure to xenobiotic agents has been proposed as a key factor influencing the composition and function of the gut microbiota. Even very low doses of DEHP have been shown to disrupt the homeostasis of the gut microbiota in mice (0.1735 mg/kg/day) [20]. Furthermore, the gut microbiota composition significantly varies across different age groups due to age-related changes in immune function and other physiological factors [21]. Age plays a role in the recovery and plasticity of the gut microbiota following exposure to xenobiotics. Studies have reported a more efficient recovery of the gut microbiota in young mice compared to older mice after antibiotic treatment [21]. However, there is a notable lack of research examining the interplay between gut microbiota, human health status, DEHP exposure levels, age, and multiple organ effects.

This study aimed to assess the effects of low and high concentrations of DEHP on young and aged mice. We conducted a comprehensive evaluation of DEHP's impact on the digestive organs and gut microbiota in each group. Specifically, we examined the histological appearance and function of the stomach, liver, spleen, kidney, and ileum in the mice. Additionally, we collected and analyzed fecal samples to characterize the gut microbiota using 16S rRNA sequencing. This study provides valuable insights into the potential effects of DEHP on age-dependent changes in organ function and gut microbial composition.

2. Materials and methods

2.1. Animals and primary reagents

The 4-week-old Specific Pathogen Free (SPF)-grade C57BL/6J male mice (weighing 18–21 g) and 76-week-old SPF-grade C57BL/6J male mice (weighing 29–40 g) were purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. (Beijing, China) [License No.: SCXK (Zhe) 2019-0001]. The animals were maintained in the Animal Room, Marshall Medical Research Center, The Fifth Affiliated Hospital of Zhengzhou University. All procedures in this study were reviewed and procedures by the Animal Experimentation Ethics Committee (AEEC) of The Fifth Affiliated Hospital of Zhengzhou University (KY2021013). The corn oil and DEHP were purchased from Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China). The sterilized Feed and poplar shavings were purchased from Jiangsu Synergy Pharmaceutical Bioengineering Co., Ltd. [Su Feed Certificate (2019) 01008]. The alkaline phosphatase detection kit was purchased from Shenzhen Kubel Biotechnology Co., Ltd (Shenzhen, China).

2.2. Experimental design

All experiments were performed in compliance with the guidelines of the Ethics Committee of Zhengzhou University. The 29 young male C57BL/6J mice aged at four weeks (19–21 g) and 20 male 76 weeks old C57BL/6J mice (29–40 g) were bred in a controlled environment (a temperature of 22 °C, a 12 h/12 h light/dark cycle and with *ad libitum* access to food and water) for seven days, referring to the Liting Zhou's research [22]. The animals at each life stage were randomly divided into three groups, based the previous studies, including a control group (0 mg/kg·bw), low dose (50 mg/kg·bw), and high dose (500 mg/kg·bw) of DEHP [22–27]. The DEHP was administered to the mice daily for a period of 12 weeks by adding it to the corn oil. Throughout this 12-week period, several parameters were measured and recorded. The body weight of the mice and their food intake were measured on a weekly basis. Additionally, the mice's hair gloss, activity level, and post-gavage state were assessed daily. Fecal samples were collected from the mice and stored in sterile EP (Eppendorf) tubes at −80 °C for further analysis. Blood samples were obtained from each mouse's eyeball. After sacrifice, samples of the stomach, liver, and spleen were quickly dissected and weighed. The liver and spleen indices were calculated using the formula: tissue index (%) = wet tissue weight/Mouse body weight × 100 %.

2.2.1. Biochemical analysis of blood

After the 12-week gavage period, the mice were euthanized following a fasting period of over 12 h to empty their feces. During this fasting period, the mice had access to water but no food. To ensure complete anesthesia, the mice were anesthetized with isopentane. Once the mice were fully anesthetized, their right eye was gently squeezed to protrude the eyeball. Blood was collected using ophthalmic forceps and transferred into a high-pressure sterilized 1.5 mL EP tube. The blood samples were kept at room temperature for 1 h and then centrifuged at 5000×*g* using an Allegra V-15R centrifuge (Thermo, USA) for 15 min. After centrifuge, the supernatants were transferred to sterile EP tubes. Liver function, including the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as renal function, including creatinine (Cr) and UREA levels, were determined using an

automatic biochemical analyzer (iMagic-V7, Shenzhen Kubeier Biotechnology Co., Ltd.) and commercially available assay kits from the same company. The levels of diamine oxidase (DAO) and D-lactic acid (LA) were detected using ELISA and a colorimetric method, respectively, with commercially available kits from Wuhan Ilarite Biotechnology Co., Ltd. All biochemical analyses were performed following the manufacturer's instructions.

2.2.2. Histological analysis

The fresh liver, spleen, and ileum samples of mice were fixed with 4 % paraformaldehyde overnight and dehydrated, embedded, sliced, spread, removed, baked, and stained with hematoxylin and eosin (HE) solution. Structural changes and inflammation of the tissues were observed and record under a light microscope (Leica Microsystems, Shanghai) [28].

2.2.3. 16S rRNA sequencing and analysis

Total microbial DNA was extracted using the DNA extraction kit (Tiangen Biotechnology, Shanghai, China). The integrity, concentration, and purity of DNA were detected before sequencing on the Illumina NovaSeq 6000 platform (Majorbio, Shanghai). Illumina PE300 reads were obtained by sequencing the V3–V4 region of the 16S rRNA gene. The primer pair were 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTAC HVGGGTW TCTAAT-3′). PCR was conducted on an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). After splicing, QIIME2 (2022.2) was used to clean the raw reads, and the samples with 16S rRNA sequence similarity over 97 % were defined as an operational taxonomic unit (OTU). OTU clustering and species annotation were then conducted using SILVA ribosomal RNA database (https://www.arb-silva.de/). Based on OTU cluster analysis, statistical and visual analysis of gut microbiota at the phylum and genus levels were performed with R studio (4.1.3). To compare the relative abundance of significantly different taxa in the gut microbiota, the researchers employed Linear Discriminant Analysis Effect Size (LEfSe). LEfSe is a statistical method that utilizes nonparametric tests, such as the Wilcoxon rank sum test for comparing two groups and the Kruskal-Wallis test for comparing more than two groups. An LDA value > 2 was considered an essential contributor to the model.

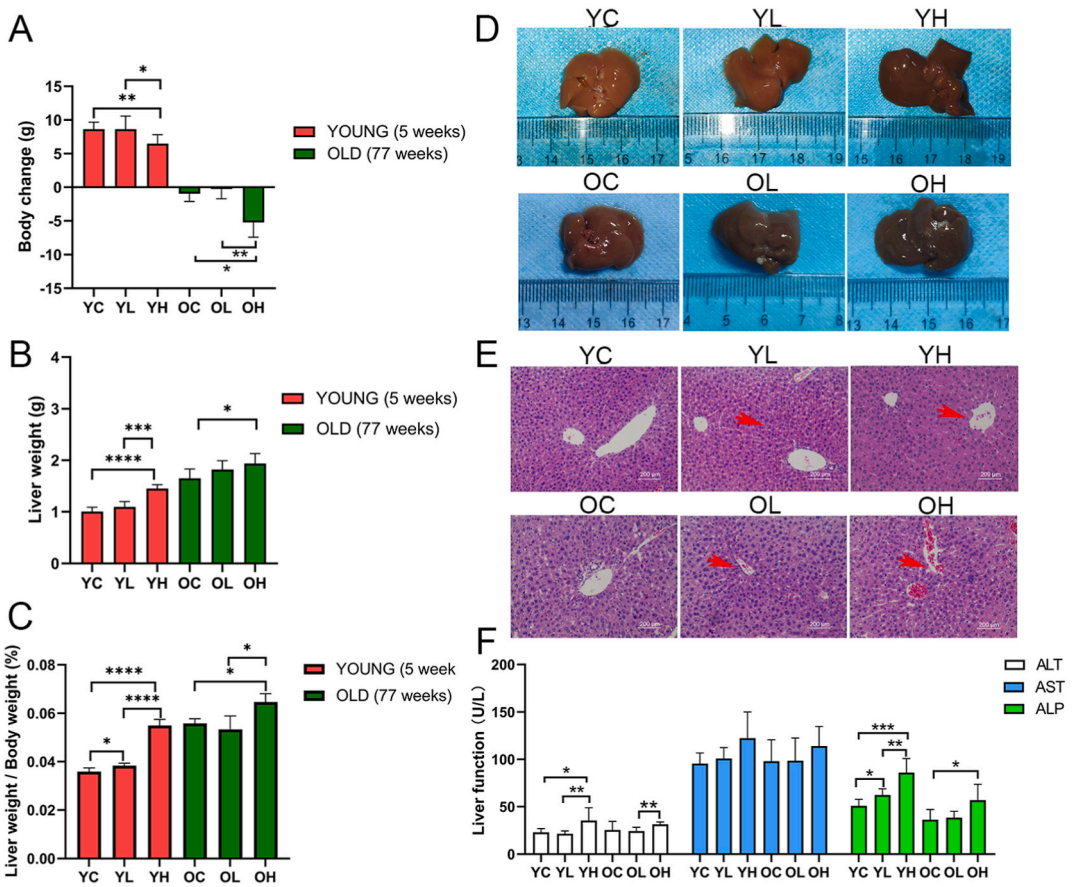

Fig. 1. Body and liver weight of mice under different doses of DEHP for 12 weeks. (A) Body weight, (B) Liver weight, (C) the Liver body weight ratio, (D) appearance of hepatic tissue, (E) representative photomicrographs of hepatic tissue (H&E staining; magnification × 200), and (F) liver function of mice in each group at 12 weeks. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. The arrow indicates the site of injury.

2.3. Statistical analysis

The data were presented as the mean ± SEM or SD. Data analysis was performed using SPSS software (version 22.0) with a one-way ANOVA. In the statistical analysis, * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and **** indicates $P < 0.0001$. Graphs were generated using GraphPad Prism software (version 8.0).

3. Results

3.1. Weight loss and liver damage

The mice in the high-dose group exhibited signs of sluggishness, lethargy, and decreased vitality. After 12 weeks of DEHP exposure, the body weight increased in the young group but decreased in the old group (Fig. 1A). Furthermore, compared with the control group, both the young (YL, $P > 0.05$; YH, $P < 0.01$) and aged (OL, $P > 0.05$; OH, $P < 0.05$) groups showed increased liver weights as exposed to DEHP at either low or high doses (Fig. 1B). In young mice, the liver-to-body weight ratio increased in a dose-dependent manner. However, in the aged group, this ratio significantly increased only in mice exposed to a high DEHP dose (Fig. 1C, $P < 0.001$).

The liver color became darker as the concentration of DEHP increased in both the young and old groups (Fig. 1D). HE analysis of the liver in the YC group revealed a normal cellular and histological structure. However, mice exposed to DEHP exhibited various pathological changes. In the YL and YH groups, the liver showed slight dilation and congestion. In the OL group, inflammatory cell infiltration was observed along with unclear cell boundaries. In the OH group, both inflammatory cell infiltration and blood vessel congestion were observed (Fig. 1E).

In addition, DEHP exposure significantly increased ALT and ALP enzymatic levels in the YH and OH group (YH, $P_{ALT} < 0.01$, $P_{ALP} < 0.001$; OH， $P_{ALT} < 0.01$, $P_{ALP} < 0.05$) (Fig. 1F), which suggest that DEHP exposure had a greater impact on the liver in the young group compared to the old group.

3.2. Inflammation in the spleen of mice

To determine the DEHP effects on the spleen, we weighed the mice spleens after treating them with different concentrations of DEHP. Compared with the YC and OC groups, the spleen weight of the YH and OH groups significantly increased ($P < 0.05$ and $P < 0.01$), respectively (Fig. 2A). The analysis showed an increased spleen index in young and old mice in a dose-dependent manner. Similarly, the spleen index of the YH group was significantly increased than the YC group ($P < 0.01$) and YL group ($P < 0.01$). In the

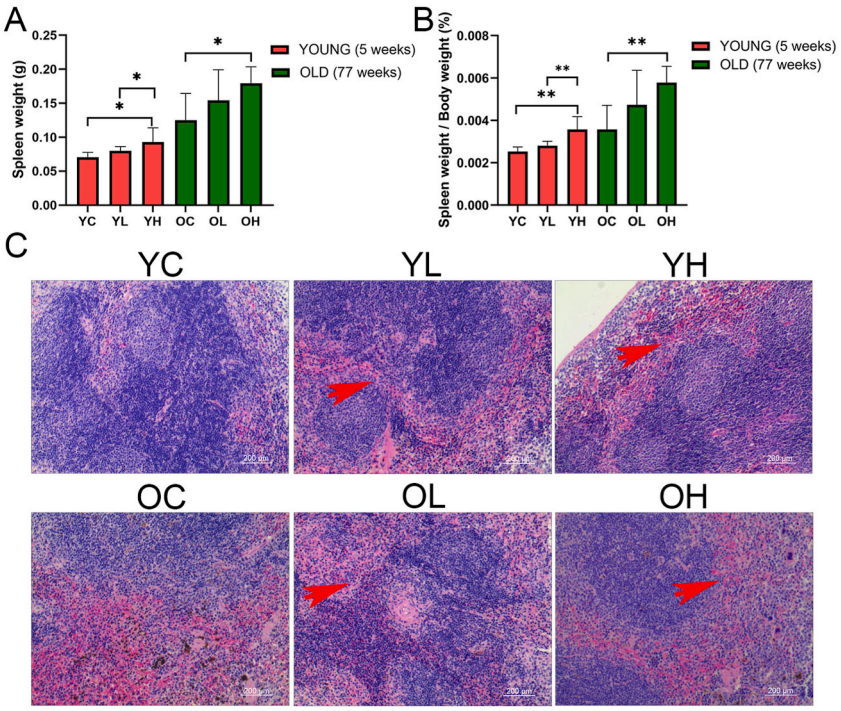

Fig. 2. Spleen damage of mice exposed to different doses of DEHP at different ages for 12 weeks. (A) Spleen weight, (B) Spleen index, and (C) Representative photomicrographs of H&E staining (magnification, × 200) of the spleen of mice in each group at 12 weeks. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. The arrow indicates the site of injury.

aged mice, the OH group was significantly higher than the OC group ($P < 0.001$) (Fig. 2B), while the appearance of spleen showed no significant difference in each group (Fig. S1). HE staining showed substantial recruitment of the inflammatory cells in the spleen of mice in each group after a gradual increase in the DEHP dose. In addition, the YL group exhibited some inflammatory cells in the cortical and paracortical areas. In the YH group, there was a higher infiltration of inflammatory cells in the cortex, and the lymphatic follicular structure appeared unclear. In the OL group, inflammatory cells infiltrated the red pulp, and the white pulp structure showed irregularities. Furthermore, there was a higher presence of macrophages in the OH group compared to the OL group (Fig. 2C), indicating that DEHP induced dose-dependent inflammation in the spleen. However, the HE staining results of the gastric tissue in both the young and old groups did not show any noticeable damage (Fig. S2).

3.3. DEHP-induced renal structural disorder and inflammatory cell infiltration in mice

The histopathological examination of the kidneys revealed an increased presence of inflammatory cells in each experimental group. The kidneys of the YC group did not show any obvious abnormalities, but there was an increase in myelitis cells in the YL group. In the YH group, the renal tubules exhibited distorted and twisted shapes, along with the presence of numerous inflammatory cells and bleeding in the glomeruli. The structure of the OC group showed partial disruption, and the blood vessels were slightly congested. In the OL group, the kidney structure appeared normal, with scattered infiltration of inflammatory cells. In the OH group, inflammatory cells were also observed but scattered (Fig. 3A), indicating that DEHP induced an inflammatory reaction in the kidneys. Furthermore, compared with the control group, the YH and OH groups exhibited a significant increase in the CR ($P < 0.05$), and the YL, YH, and OH groups showed a significant increase in urea levels ($P < 0.05$) (Fig. 3B).

3.4. DEHP caused the rupture of ileum villi in mice

Compared with the YC and OC, the ileum villi were fracted in young and old mice under different doses of DEHP exposure (Fig. 4A). Subsequently, the intestinal function of mice in each group was further studied. The levels of DAO and D-lactate were increased in young and old mice ($P < 0.05$, Fig. 4B and C); moreover, the increase of serum DAO levels from the OH group and OC group was significantly different ($P < 0.05$, Fig. 4B).

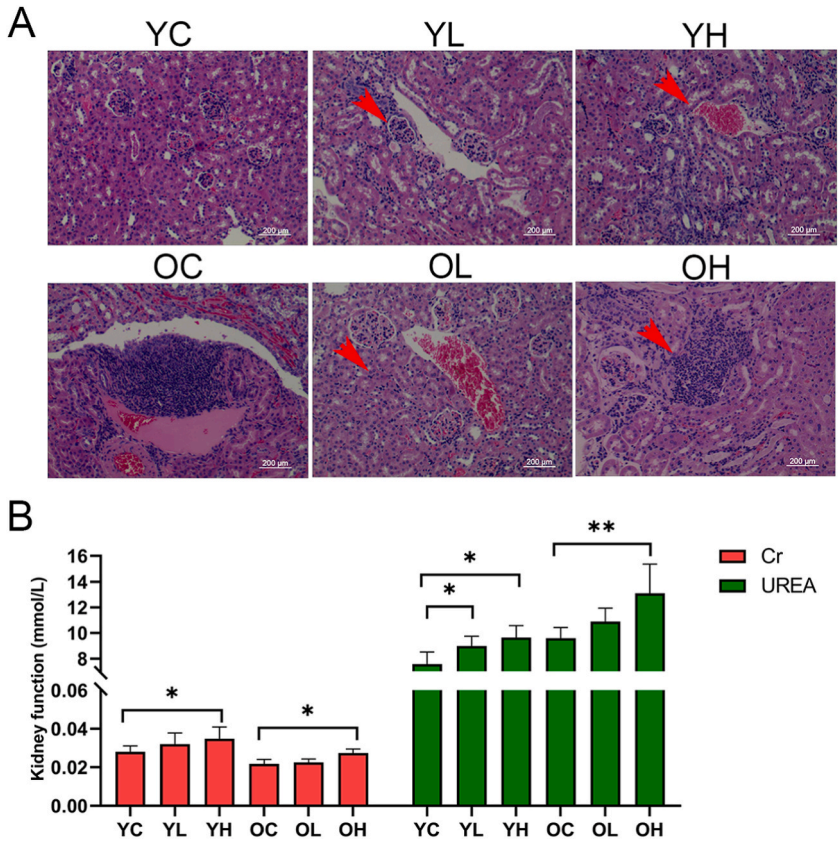

Fig. 3. Kidney damage of mice exposed to different doses of DEHP at different ages for 12 weeks. (A) H&E staining of renal tissue (magnification, × 200) and (B) kidney index of mice in each group at 12 weeks. *$P < 0.05$, **$P < 0.01$. The arrow indicates the site of injury.

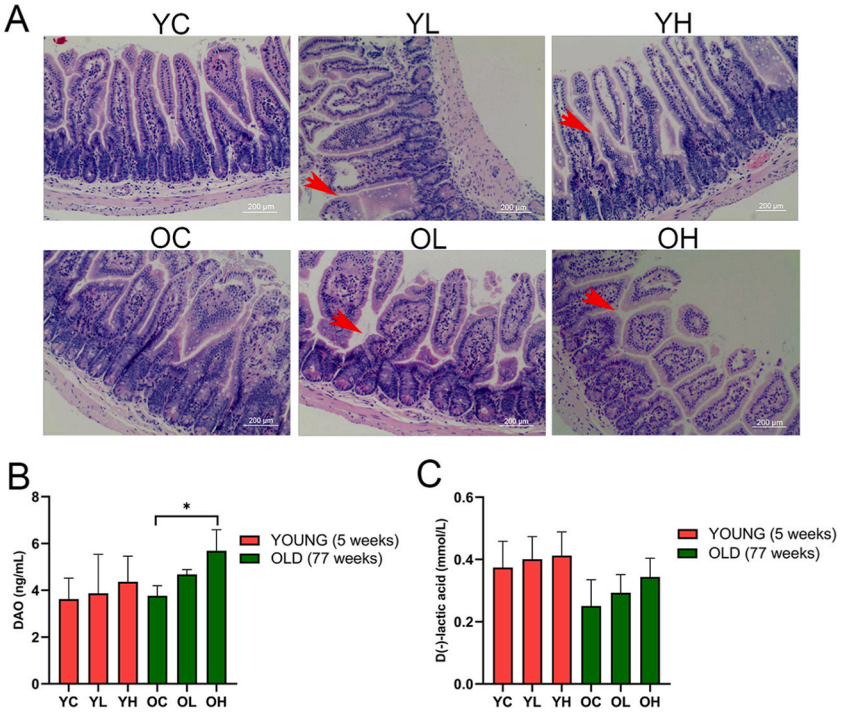

Fig. 4. Ileum damage of mice exposed to different doses of DEHP at different ages for 12 weeks. (A) H&E staining of the ileum (magnification, × 200), (B) Serum DAO, and (C) Serum D-lactate level of mice. *$P < 0.05$. The arrow indicates the site of injury.

3.5. DEHP disrupted the homeostasis of gut microbiota in mice

Considering the observed liver damage caused by DEHP exposure in mice, it was hypothesized that the gut microbiota might also be affected due to disruptions in the "gut-liver axis." To investigate this, gut microbial 16s rRNA sequencing was conducted on a total of 49 samples from six different groups. After quality filtering, the resulting clean reads were extracted and utilized for further analysis. The α-Diversity measures, including observed genes, Shannon index, Simpson index, ACE index, and Chao index, were assessed to evaluate the richness and evenness of the gut microbiota. Additionally, β-Diversity analysis was performed to examine the taxonomic differences among the various experimental groups. Interestingly, no significant differences were observed in α-Diversity, indicating that the abundance and evenness of the gut microbiota were comparable among the different groups (Fig. S3, Fig. 5A and 5B). At the phylum level, the relative abundance of Firmicutes showed a gradual decrease with increasing DEHP dose in the young group (Firmicutes: 50.9 % in YC, 44.3 % in YL, and 39 % in YH), while the abundance of Bacteroidetes showed an opposite trend with increasing DEHP dose (Bacteroidetes: 24.8 % in YC, 35.9 % in YL, and 38.4 % in YH) (Fig. 5C). In contrast, the relative abundance of Firmicutes and Bacteroidetes in the old group showed the opposite pattern (Firmicutes: 61.3 % in OC, 36.8 % in OL, and 32.4 % in OH; Bacteroidetes: 28.6 % in OC, 37.7 % in OL, and 43.9 % in OH) (Fig. 5D). Compared with the control groups, the ratio of Firmicute/Bacteroidetes was gradually decreased in a dose-dependent manner in young and old mice (Fig. 5E and F, YH vs. YC, $P < 0.05$; OL vs. OC, $P < 0.05$).

The structural composition of microbiota in each group at the genus level was also analyzed (Fig. 6). *Bifidobacterium* and *Muribaculum* were the dominant microbiota in all groups (Fig. 6A and B). In the YC group, *Barnesiella*, *Bifidobacterium*, and *Allobaculum* were the dominant genera. Compared with the YC group, the relative abundance of *Deslfovibrio*, *Olsenella*, *Enterrhabdus*, *Deslfovibrio*, *Turicibacter*, *Muribaculum*, and *Clostridia*_UCG-014 were significantly enriched in the YL group (Fig. 6C). In parallel, *Muribaculum*, *Dubosiella* and *Ileibacterium* in the YH group were enriched (Fig. 6D). In parallel, the YH group significantly decreased *Alistipes*, *Mucispirilum*, and *Staphylococcus* showed a significantly decreased in the YH group, while *Bifidobacterium*, *Muribaculum*, and *Dubosiella* were enriched considerably.

Lactobacillus was the most abundant bacteria in the OC group. The abundance of *Enterococcus*, *Staphylococcus*, and *Lactobacillus* in the OL group was significantly decreased (Fig. 6E). Compared with the OC group, the amount of *Odoribacter*, *Staphylococcus*, and *Enterococcus* was reduced considerably, whereas the amount of *Bifidobacterium*, *Dubosiella*, and *Deslfovibrio* was significantly enriched in the OH group (Fig. 6F).

4. Discussion

In this study, we initially compared the effects of DEHP exposure on multiple organs in young and old mice. We observed various

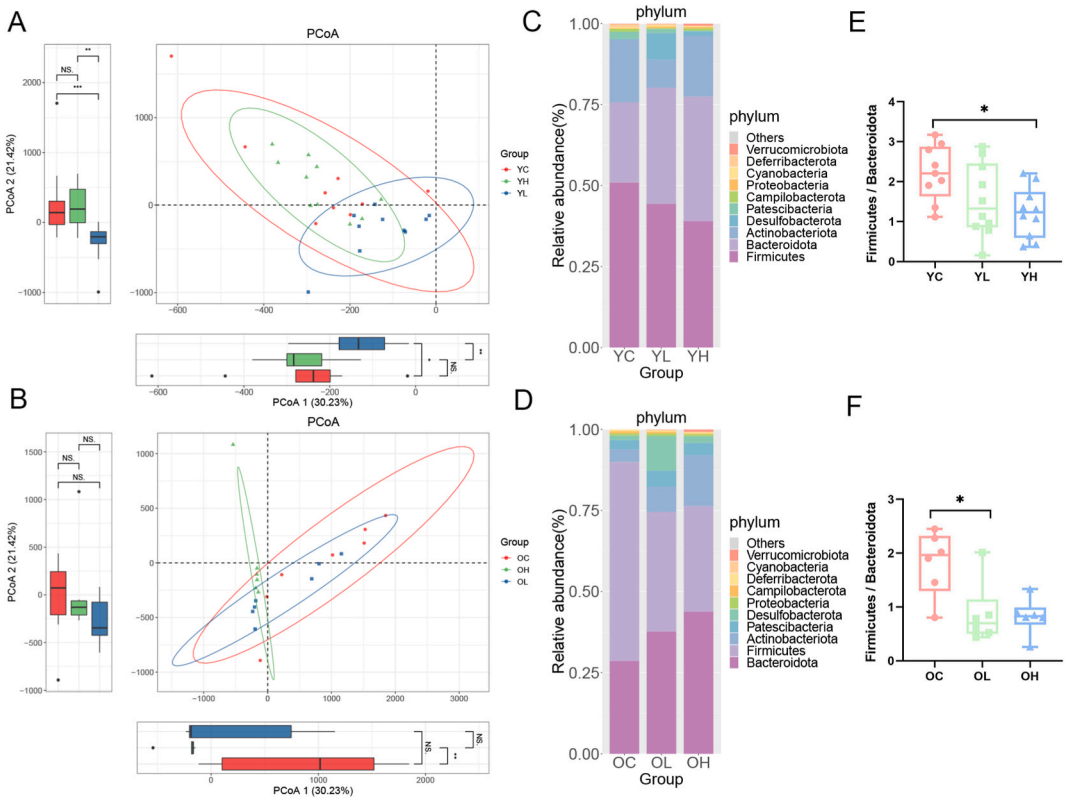

Fig. 5. Distribution and composition of gut microbiota of mice exposed to DEHP at a different ages for 12 weeks. Principal coordinate analysis (PCoA) of genus level in (A) young group and (B) old group. Taxonomic composition at the phylum level in (C) young and (D) old groups. The ratio of Firmicutes and Bacteroidetes in the gut microbiota of (E) young and (F) old groups.

damages, including changes in liver texture and cellular/histological morphology, spleen inflammation, renal inflammatory cell infiltration, and intestinal villus rupture. Mice in the high-dose DEHP group exhibited sluggish movement, decreased vigor, poor mental state, reduced glossiness of back hair, and significant weight loss. Interestingly, the weight loss in old mice was more pronounced than in young mice under the same DEHP exposure, likely due to reduced dietary intake. We found that DEHP exposure led to an increase in liver weight, consistent with previous findings in Largemouth bass. Moreover, DEHP has been reported to cause hepatomegaly and hepatic injury. Notably, within just three weeks of DEHP exposure, we observed a gradual increase in renal inflammatory cells and structural abnormalities in the liver and spleen of Sprague-Dawley rats and 3-week-old ICR mice. Interestingly, while inflammation in the liver was relatively low, spleen and kidney inflammation were more severe in old mice, possibly due to differences in their gut microbiota composition. Histological analysis of the ileum revealed mild villus rupture in both young and old mice, similar to findings in 4-week-old C57BL6J mice. Additionally, we observed an increasing trend in the activity of diamine oxidase (DAO) and levels of D-lactate, which are well-known markers of intestinal barrier impairment, in all mice exposed to DEHP. DAO is an enzyme essential for maintaining intestinal mucosal integrity, and increased intestinal permeability leads to elevated serum levels of D-lactate, a metabolite of gut microbiota. The presence of ruptured villi, elevated DAO activity, and increased D-lactate levels suggest that DEHP exposure disrupts the intestinal barrier, facilitating the entry of harmful substances, including toxins and bacteria.

The liver is the crucial organ that decomposes and metabolizes toxic substances and prevents bacteria-derived metabolites from entering the blood circulation to harm the body [29]. Once the liver function is damaged, the gut microbiota can significantly change as an essential part of the liver-gut axis [30]. In this study, the gut microbiota was considerably altered, accompanied by liver damage. 16s rRNA sequencing analysis of mice feces showed that DEHP exposure reduced the diversity and abundance of gut microbiota. Although the previous studies found the gut microbiota dysregulation from 4-week-old C57BL6J mice caused by DEHP exposure [12], we revealed this pattern at ages ranging to 12 weeks. Notably, we found that the gut microbiota of old mice can also be dysregulated under the intervention of DEHP. Previous studies have shown that Firmicute/Bacteroidota ratio positively correlates with mammalian body weight [12], further confirmed in this work from young and old mice. At the genus level, the gut microbiota changes were variable between young and aged mice exposed to DEHP.

In the young mice group, the YC group enriched different beneficial microbiota, such as specific probiotics Bifidobacterium, Bacteroides, and short-chain fatty acids (SCFAs) producing microbiota *Lachnospiraceae*_UCG-006, *Enterrhabdus* [31,32], *Faecalibaculum*, and *Alistipes* [33,34]. *Bifidobacterium* helps to decompose proteins, lipids and some complex polysaccharides in the gut and can alleviate the metabolites of inflammatory development. It maintains intestinal homeostasis and provides exceptional protection for the

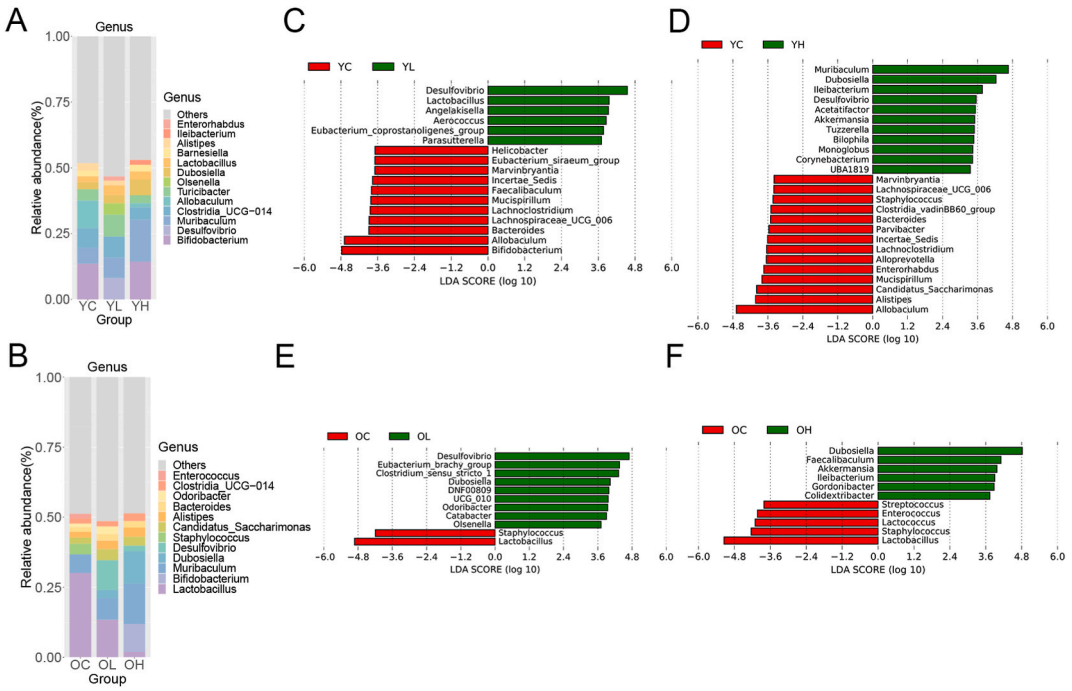

Fig. 6. The gut microbiota of mice exposed to different DEHP doses to varying ages by 16S rRNA gene sequencing. Taxonomic composition at the genus level in (A) young and (B) old groups. LDA value distribution histogram of (C) YC and YL groups, (D) YC and YH groups, (E) OC and OL groups, and (F) OC and OH groups.

gut to prevent the body from being damaged by invasive pathogens [35]. Some gut microbiota obtain carbon and energy through the hydrolysis of carbohydrate molecules, providing a certain degree of protection for the gut [36]. SCFAs can maintain the normal function of the large intestine, promote sodium and calcium absorption, increase intestinal functions, promote digestion, repair the damaged intestinal mucosa, and participate in the host's energy metabolism through the transformation of the liver [37]. However, exposure to DEHP (YL and YH groups), the SCFAs-producing bacteria *Lachnospiraceae*_UCG-006, *Enterorhabdus*, *Faecalibaculum*, and *Alistipes* in the YC group significantly declined. Additionally, the YL group's dominant bacteria, such as Desulfovibrio, Aerococcus, Muribaculum, and Corynebacterium, were primarily pathogenic. *Desulfovibrio* can produce lipopolysaccharide and hydrogen sulfide, leading to intestinal barrier damage, butyrate oxidation and cell apoptosis [38]. *Aerococcus* can cause urinary tract infections, blood flow infections, endocarditis, bone and joint infection, and soft tissue infection [39]. The high abundance of *Muribaculum* may be related to intestinal inflammation [40]. *Corynebacterium* is increasingly thought to cause disease when immune function is low [41]. Therefore, the alteration of gut microbiota (as indicated by the increase in pathogenic bacteria and the decrease in beneficial bacteria) may be the factor that leads to liver damage and villi rupture in the YL and YH groups.

Regarding the old mice group, the OC group showed dominance of *Lactobacillus* and *Staphylococcus* bacterial groups, which can cause various diseases in humans and animals through toxin production or penetration [42]. In comparison to the OC group, the OL group and OH group exhibited a significant decrease in *Lactobacillus* abundance, along with a dramatic increase in *Desulfovibrio* and *Dubosiella*, respectively. Studies have indicated higher abundances of *Dubosiella* and *Desulfovibrio* in mice with liver injury [43–45]. Importantly, high-dose DEHP exposure increased the presence of pathogenic bacteria in the gut while significantly increasing beneficial bacteria in the guts of the old group, such as *Dubosiella*, *Akkermansia*, and *Monolobus*. These bacteria may contribute to improved intestinal immunity, maintenance of intestinal barrier function, and enhanced anti-inflammatory activity [46–48]. This may partially explain the milder liver damage observed in the aged mice exposed to DEHP compared to young mice (Fig. 1E). The intervention period for the mice in this study lasted almost three months, making it challenging to determine the specific changes occurring in each organ and the intestinal microbiota. Although the limitations in our model's measurements reduce its explanatory power, it can be speculated that supplementation with *Lactobacillus* may offer protection against intestinal injury caused by DEHP exposure [49].

In conclusion, this study provides first evidence that 12 weeks of DEHP exposure results in organ damage (liver, kidney, and ileum) and gut microbiota dysbiosis in young and aged mice, with varying degrees of severity. These findings offer insights into the impact of DEHP on organ damage and gut microbiota homeostasis in different age groups, thus establishing a valuable model system for understanding the effects of DEHP on human health. This study fills the research gap regarding the elderly model system and lays the foundation for developing microbial interventions to restore gut health.

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Ethics approval

All procedures in this study were reviewed and procedures by the Animal Experimentation Ethics Committee (AEEC) of The Fifth Affiliated Hospita of Zhengzhou University (KY2021013).

Data availability statement

The 16sRNA data are available at NCBI (BioSample accessions are SAMN35441442-SAMN35441500, which are included in BioProject: PRJNA976758, https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA976758). Our analyses' protocols and raw figures or other information related to our study could be asked from the corresponding author on reasonable request.

CRediT authorship contribution statement

Qiang Zhang: Data curation. **Chunjing Qiu:** Writing – original draft. **Wenya Jiang:** Writing – original draft. **Pengya Feng:** Writing – review & editing. **Xia Xue:** Writing – review & editing. **Ihtisham Bukhari:** Writing – review & editing. **Yang Mi:** Conceptualization. **Pengyuan Zheng:** Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22677.

Abbreviations

DEHP	Dioctyl phthalate, di 2-Ethylhexyl phthalate
SPF	Specific Pathogen Free
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ALP	alkaline phosphatase
Cr	creatinine
DAO	diamine oxidase
LA	D-lactic acid
HE	hematoxylin and eosin
OTU	operational taxonomic unit
LDA	Linear Discriminant Analysis
LEfSe	Linear Discriminant Analysis effect size

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