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RESEARCH ARTICLE

Regulation of Microbiota by Morel Polysaccharide Alleviates Small Intestine Damage in LPS-Induced Mice

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ABSTRACT

This study investigates the effect of Morel's polysaccharides (MP) against LPS-induced intestinal damage by regulating oxidative stress, inflammation, and microbiota, compared with previous studies that have examined the protective roles of polysaccharides or focused solely on LPS-induced intestinal damage in mice. Thirty (30) ICR mice were grouped into AC (control), AM (LPS model), and AT (treatment) groups. Group AT received MP treatment for 20 days, followed by LPS treatment for 24 hours intraperitoneally, while group AM only received LPS. Results indicated a slight weight gain in group AT treated with MP compared to other groups. Conversely, significant weight loss was observed in mice of group AM following LPS treatment ($P<0.0001$). Pathological analysis demonstrated that LPS severely interfered with the integrity of intestinal villi, with group AC and group AT exhibiting significantly longer villus height than group AM ($P<0.0001$). Serum analysis indicated that TNF-α and MDA levels were significantly higher in group AM ($P<0.05$), but IL-10 ($P<0.001$), NO ($P<0.01$), SOD ($P<0.01$), and GSH-Px ($P<0.0001$) were significantly lower in LPS-induced ICR animals in group M. The genus of *Erysipelatoclostridiaceae* was higher in group AC, while *Proteobacteria*, *Enterobacteriaceae*, *Enterobacterales*, *Gammaproteobacteria*, *Escherichia*, *Shigella*, *Enterococcus*, and *Enterococcaceae* were higher in group AM. Group AT exhibited higher levels of *Peptococcus*, ASF356, *Roseburia*, unclassified *Prevotellaceae*, *Acetatifactor*, *Vampirivibrionia*, and *Cyanobacteria*. The results show that MP treatment significantly enhanced antioxidant enzyme activities, reduced oxidative stress markers, and improved overall oxidative resistance compared to the LPS group. MP also mitigated inflammation and favorably modulated the gut microbiota, which suggests its potential as a therapeutic agent for intestinal health.

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INTRODUCTION

Morels, well-known for their nutritional value, have a rich history in traditional Chinese medicine, documented for over 400 years (Xu *et al.,* 2022; Ren *et al.,* 2023). Previous studies reported that morels possess many functions, including oxidation resistance, immunoregulation, and anti-cancer (Tang *et al.,* 2019; Wen *et al.,* 2019; Xiong *et al.,* 2021). Polysaccharides, comprising over ten monosaccharides, are widely known carbohydrate polymers with a crucial role in promoting the structural integrity and energy storage of the host (Huang *et al.,* 2018; Anwar *et al.,* 2023). Hypotoxic natural polysaccharides, polysaccharides derived from natural sources and considered non-toxic to biological systems, are known for immuno-enhancing, oxidation resistance, and blood-regulating functions, and play pivotal roles in maintaining overall health (Wen *et al.,* 2019; Wang *et al.,* 2020; Ahmad *et al.,* 2023). Morels, being polysaccharide-rich fungi, have been found to exhibit various beneficial effects such as promoting immune activity because of their inherent ability to stimulate immune cell proliferation and cytokine production, thereby enhancing immune function (Fehily *et al.,* 2021). Besides, they are associated with antioxidant capacity, anti-bacterial properties, liver protection, and anticancer activities (Xiong *et al.,* 2021). Morel polysaccharides also act as a prebiotic, promoting the growth and activity of beneficial gut microbiota and

enhancing microbial diversity within the gut environment (Jayachandran *et al.,* 2019), besides their assumed role in the maintenance of intestinal barrier integrity, protecting against intestinal damage induced by external insults, thereby suggesting its potential utility in preserving gut health (Ferreira *et al.,* 2021).

The complex connection between the host and its gut microbiota contributes extensively to maintaining intestinal homeostasis and overall health (Zafar *et al*., 2019). The gut microbiota, comprising millions of organisms, such as microbes, fungi, viruses, protozoa, and archaea (El-Sayed *et al.,* 2021; Kirn *et al.,* 2023), play essential roles in organism metabolism, immunoregulation, and intestinal barrier function (El-Sayed *et al.,* 2021; Jia *et al.,* 2021). Dysregulation of flora is associated with conditions such as ulcerative colitis (Meng *et al.,* 2020), colorectal cancer (Fan *et al.,* 2021), inflammatory bowel disease (Thursby and Juge, 2017), and diarrhea (Nawaz *et al*., 2020; Li *et al.,* 2021). Building on the foundational roles of the gut microbiota in host physiology, recent research has turned attention to specific dietary components, such as Morel polysaccharides (MP), which may exert targeted modulatory effects on microbial composition and function.

Lipopolysaccharides (LPS) are known to induce severe inflammation and oxidative stress in the intestines, leading to damage and dysfunction. Gram-negative bacteria, widely distributed in microbiota, contain lipopolysaccharide as a crucial component (Wassenaar *et al.,* 2018; Gao *et al.,* 2023). LPS acts as a potent endotoxin, which is associated with various diseases, including intestinal inflammation, pneumonia, liver injury, and diabetes mellitus (Zhou *et al.,* 2022). LPS is also well-known for its role in stimulating immunoreaction and the secretion of gut phlegmonosis in experimental animals like mice, chicks, and pigs (Li *et al.,* 2020a; Fu *et al.,* 2021). Morel polysaccharides derived from *Morchella esculenta* have shown promise in modulating oxidative stress and inflammation. However, there is limited information regarding the impact of MP on the gut flora of mice induced by LPS. Consequently, this investigation was conducted to explore the potential mitigating effect of MP on gut damage caused by LPS through microbiota analysis. The small intestine has the primary role in nutrient absorption and significant exposure to LPS-induced damage, making it a critical site for studying the protective effects of MP. By explicating the mechanisms underlying the potential therapeutic and protective effects of MP against LPS-induced minor intestine damage, this research may contribute to the development of novel therapeutic strategies for alleviating intestinal inflammatory diseases.

MATERIALS AND METHODS

Extraction of polysaccharides: Morels were purchased from Henan Wannong Biotechnology (Nanyang, China), washed, air-dried, crushed into a fine powder using a mortar and pestle, and filtered through 100-mesh sieves. The extraction of polysaccharides from morels followed established procedures using a 70% ethanol solution, at a temperature of 80°C for 2 hours, as described by Huo *et al.* (2020). The extract was then filtered, concentrated, and lyophilized. The concentration of Morel polysaccharide (MP) was measured using a spectrophotometric method, involving an improved phenol-sulfuric acid technique as outlined in a prior study by Tang *et al.* (2018).

Animals: ICR mice (n=30), aged five weeks (24.68±0.74 g) of both sexes were procured from Qinglong Mountain Mice Reproduction (China). A three-day acclimatization period preceded the random grouping of mice into AC, AM, and AT groups. Group AT received MP (100 mg/kg) by gavage, while groups AC and AM were administered an equal volume of double-distilled water for 20 days. On the 21st day, mice in groups AM and AT were injected with LPS (10 mg/kg) (Solarbio Science & Technology, Beijing, China) intraperitoneally. Twenty-four hours later, all animals were euthanized using carbon dioxide to collect serum and intestinal samples. Blood was collected via cardiac puncture and processed by centrifugation at 3000 rpm for 10 minutes to obtain serum, which was then stored at 4°C for further analysis.

Hematoxylin & Eosin Staining: Small intestinal tissue samples collected from all animals were preserved in 4% paraformaldehyde for 48 hours. Following fixation, the tissues underwent sequential dehydration using increasing concentrations of ethanol, were cleared in xylene, and then embedded in paraffin blocks. Using a microtome, thin sections of 5 µm were prepared and affixed onto glass slides for Hematoxylin and Eosin staining (Pinuofei Biological, China). The stained sections were examined under an Olympus BX46 microscope (Olympus, Japan) for histopathological assessment.

Nitric oxide, oxidation resistance, and inflammatory factor levels in mice: Nitric oxide (NO) levels were determined via the Griess reaction using commercial kits (Jiancheng Bioengineering Research Institute Co., Ltd, China). Serum aliquots were mixed 1:1 with Griess reagent. After 10 minutes at ambient temperature, absorbance was recorded at 540 nm using a microplate reader. NO concentrations were calculated from a sodium nitrite standard curve. Total Antioxidant Capacity (T-AOC) was assessed via a colorimetric kit (Jiancheng Bioengineering Research Institute Co., Ltd, China). Diluted samples reacted with the standardized kit antioxidants. Post-incubation chromogenic substrate reaction absorbance was measured at 405 nm. Results were normalized to Trolox equivalents and expressed as mM concentrations relative to sample volume. Oxidative stress biomarkers—malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were assessed. MDA content was determined spectrophotometrically by quantifying thiobarbituric acid-reactive substances. SOD activity was evaluated with a dedicated assay kit, while GSH-Px levels were measured using established colorimetric protocols, each following the manufacturer's guidelines. Cytokines (IL-1β, IL-6, IL-10, TNF) were measured with specific assay kits (Solarbio Life Science, China).

Microbiota analysis: Three mice per group were randomly selected for microbiome analysis to represent the typical microbial composition within each group. Total

DNA extraction was performed from caecum samples of groups of AC, AM, and AT by using magnetic soil and a stool DNA kit (Yeasen, China). DNA quality and quantification were analyzed using a Qubit (4.0) fluorometer (Invitrogen, Thermo Fisher, USA) and agarose gel (1.2%) electrophoresis. 16S rRNA (V3-V4) gene primers were used for objective gene amplification as reported previously (Chai *et al.,* 2023). Colibri ES DNA library Prep kits (Invitrogen, Thermo Fisher, USA) were utilized for library construction, and high-throughput sequencing was performed on the Illumina platform (Bioyi Biotechnology, Wuhan, China). Raw data underwent filtration using Trimmomatic (Bolger and Lohse, 2014), Cutadapt (Maki *et al.,* 2023), UCHIME (Edgar *et al.,* 2011), and USEARCH (Rognes *et al.,* 2016). Operational taxonomic units and taxonomy annotation were generated by QIIME2 (Lima *et al.,* 2021).

Statistical Analysis: Statistical data for ICR animals were collected via SPSS (27.0). Results are exhibited as mean ± SD, with significance recognized at $P < 0.05$. Alpha and beta diversity analyses were performed using QIIME2. ANOVA and linear discriminant analysis effect size were employed to discover significant taxonomic differences among ICR animals with different treatments (Yu *et al.,* 2022). Network analysis for potential correlations among bacterial taxa was conducted using R (He *et al.,* 2023).

RESULTS

Effects of MP on weight and intestinal damage in ICR animals: The concentration of MP was determined to be 36.34% by considering the dilution factor and the standard curve, which initially showed a concentration of 18.17 (Fig. 1a). While no valid difference was observed in the weekly weights of animals among different groups, group AT exhibited slightly higher weight than other groups from the 14th day to the 20th day (Fig. 1b). LPS induced significant weight loss in the AM group ($P<0.0001$), whereas MP resulted in a substantial increase in the weight of mice in the AT group (Fig. 1c). Pathological analysis revealed severe destruction of intestinal villi integrity, with significantly longer villus height in the duodenum, jejunum, and ileum in groups AC and AT compared to group AM ($P<0.0001$). The ratio of villus height/crypt depth was markedly larger in groups AC and AT compared to group AM, while crypt depth in all three intestinal parts was significantly deeper in group AM ($P<0.001$) (Fig. 2).

Effects of MP on oxidation resistance and inflammatory factors in ICR mice induced by LPS: Serum analysis showed that MP treatment enhances oxidative resistance by upregulating antioxidant enzymes like SOD ($P<0.01$) and GSH-Px ($P<0.0001$) and downregulating MDA ($P<0.05$). MP treatment reduced inflammation by inhibiting the NF-κB signaling pathway, leading to decreased levels of pro-inflammatory cytokines in group AT. While significantly higher levels of TNF-α ($P<0.05$) and MDA ($P<0.05$) in animals in the AM group, IL-10 ($P<0.001$), NO ($P<0.01$), SOD ($P<0.01$) and GSH-Px ($P<0.0001$) were markedly lower in LPS induced ICR animals in group AM (Fig. 3).

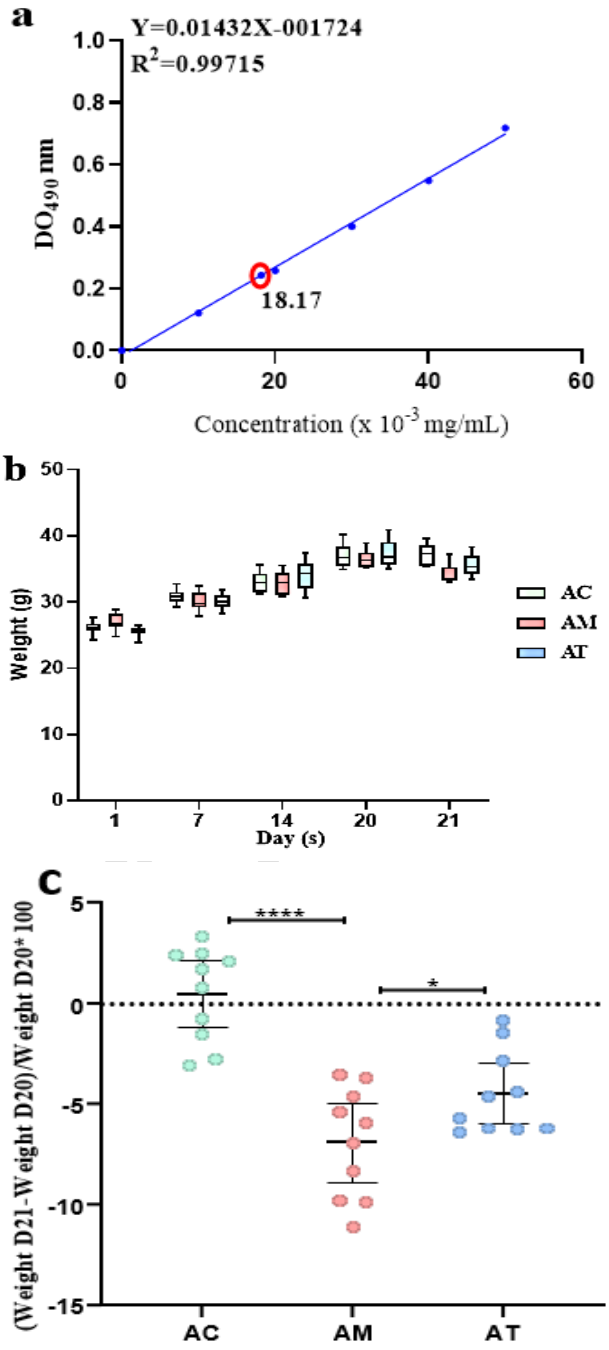

Fig. 1: The concentration of MP and its effect on the body weights of ICR animals induced by LPS. (a) Standard curve, (b) Body weights on various days, (c) Average Body weight on the 20th day. Data was presented as the mean ± SD (n=10). Significance is depicted as *P<0.05 and ****P<0.0001. Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT).

The effects of MP on the microbiota of animals induced by LPS: Raw data averaged 111,389 (AC), 83,838 (AM), and 78,574 (AT), with filtered data at 98,234 (AC), 74,323 (AM), and 68,017 (AT) (Table 1). 1843 OTUs (AC: 1 120, AM: 650, and AT: 653) were identified with 174 shared OTUs among the three mouse groups (Fig. 4a). Rarefaction and Shannon index curves indicated adequate sampling of ICR samples (Fig. 4b and 4c). Rank abundance curves were flatter, indicating high richness and evenness (Fig. 4d). No significant differences were found in alpha diversity indices (Table 2). At the phylum level, *Firmicutes* (68.75%), Bacteroidota (14.36%), and *Campylobacterota*

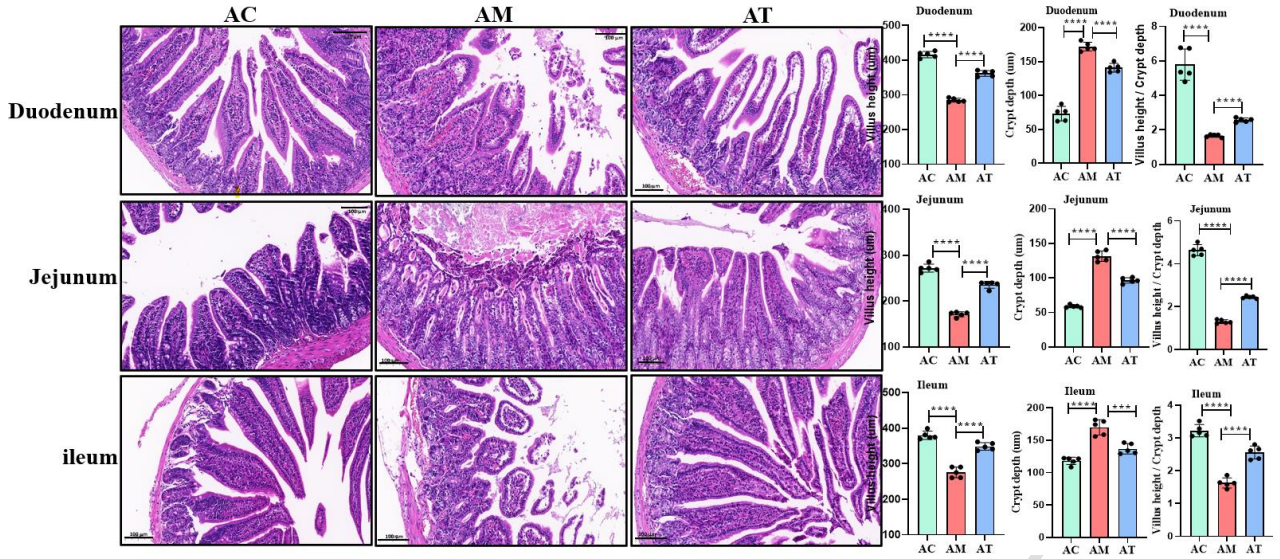

Fig. 2: The effect of MP on the intestinal damage in mice caused by LPS. Scale bar 100 μm. Significance is shown as ***P<0.001, and ****P<0.0001; data were presented as the mean ± SEM (n=5). Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT); Morel Polysaccharides (MP).

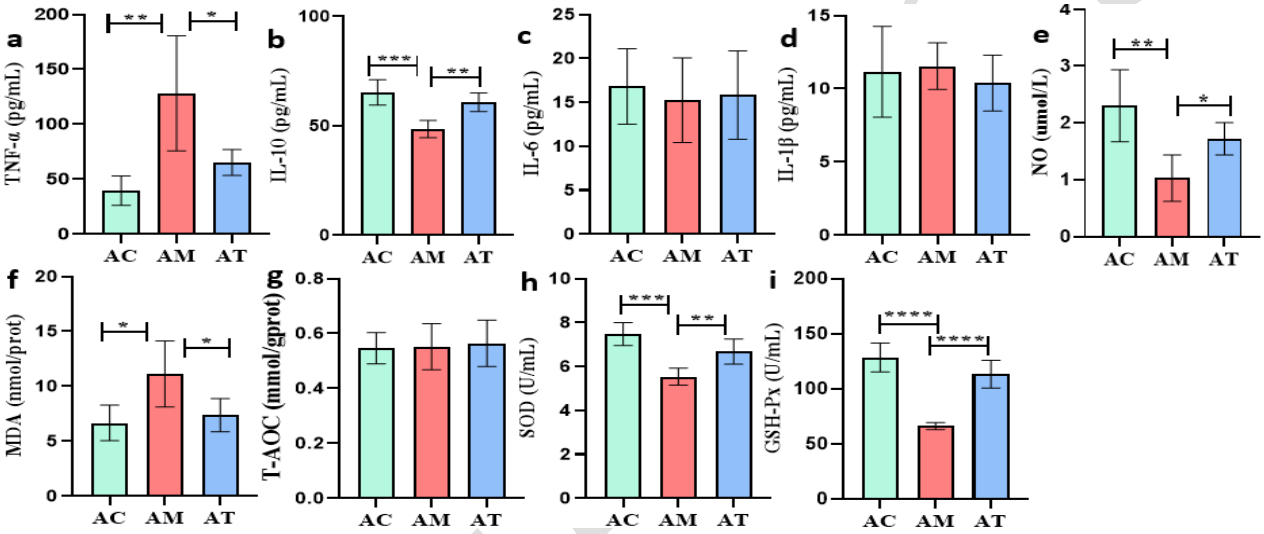

Fig. 3: The effects of MP on oxidation resistance and inflammatory factors in ICR mice induced by LPS. (a) TNF-α, (b) IL-10, (c) IL-6, (d) IL-1β, (e) Nitric oxide (NO), (f) MDA, (g) T-AOC, (h) SOD, (i) GSH-Px. Significance is denoted as *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001; data were presented as the mean ± SEM (n=5).

Table 1: Sequencing data analysis of mice in the present study.

Samples	Raw reads	Clean reads	Denoised reads	Merged reads	Non-chimeric reads
AC1	96 399	84 687	83 433	78 394	68 546
AC2	107 412	93 781	92 593	85 564	73 919
AC3	130 356	116 233	114 168	106 722	91 468
AM1	91 676	81 077	80 601	78 955	59 204
AM2	79 969	70 949	69 774	65 971	57 650
AM3	79 870	70 942	69 861	67 081	60 630
AT1	80 707	69 239	68 216	63 710	48 149
AT2	73 809	64 163	63 597	61 963	52 203
AT3	81 207	70 648	69 979	67 787	57 526

Table 2: Alpha diversity information of the microbiota of current mice

Sample	Feature	ACE	Chao1	Simpson	Shannon	PD_whole_tree
AC1	407	407.9559	407.1667	0.9728	6.5092	22.439
AC2	462	463.0387	462.6667	0.9797	6.9503	23.6161
AC3	614	616.6638	614.4375	0.9739	7.0743	35.7986
AM1	156	156.3192	156.0	0.8914	4.4247	13.9704
AM2	385	385.6531	385.0323	0.9532	6.299	25.9762
AM3	299	300.022	299.1304	0.8732	5.0238	20.8125
AT1	390	390.2574	390.0	0.985	7.0524	25.0874
AT2	235	235.9931	235.2727	0.948	5.5209	34.1108
AT3	219	219.0	219.0	0.9046	4.7365	25.31

(12.10%) predominated in group AC, while *Bacteroidota*, *Firmicutes* and *Proteobacteria* were primary phyla in group AM (38.37%, 21.48%, 34.67%) and AT (47.16%, 23.10%, 15.48%) respectively (Fig. 5a). In healthy mice (control group AC), *Firmicutes* dominated, followed by *Bacteroidota*, giving a high *Firmicutes*/*Bacteroidota* (F/B) ratio. In contrast, in LPS-induced mice (group AM), a lower F/B ratio was indicated, which is typically associated with gut dysbiosis. In the treatment (group AT), *Firmicutes* and *Bacteroidota* levels were partly restored, suggesting partial reversal of dysbiosis. At the class level, *Clostridia* (65.65%), *Bacteroidia* (14.36%) and *Campylobacteria* (12.10%) dominated in group AC, while *Bacteroidia*, *Gammaproteobacteria* and *Clostridia* were the main classes in groups AM (38.37%, 34.67%, 15.04%) and AT (47.16%, 15.48%, 16.71%), respectively (Fig. 5b). At the order level, *Lachnospirales* (50.71%), *Bacteroidales* (14.35%), and *Oscillospirales* (12.96%) were prevalent orders in group AC, while *Bacteroidales*, *Enterobacterales*, and *Oscillospirales* were mainly

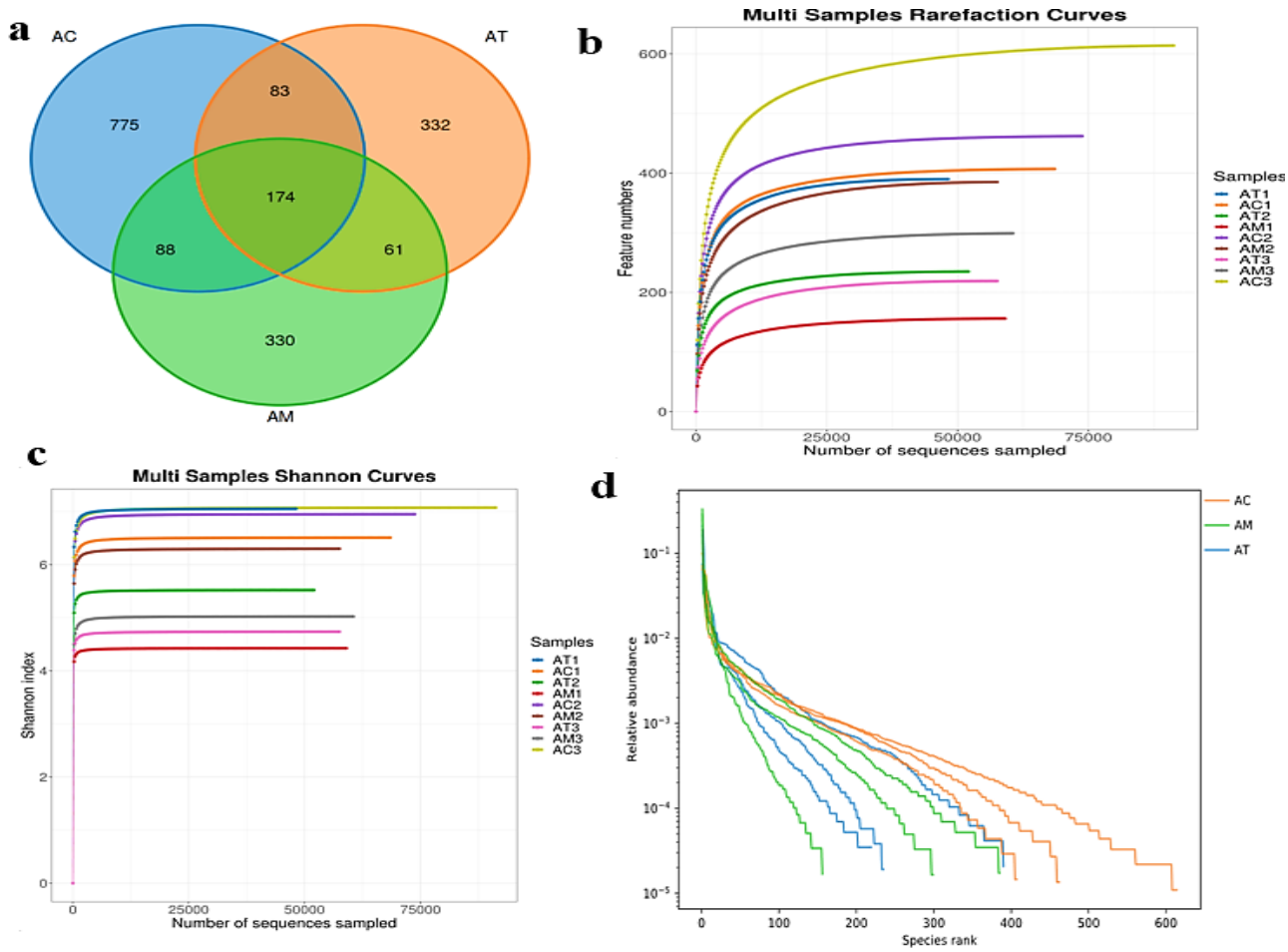

Fig. 4: The effects of MP on the microbiota structure and diversity of ICR animals induced by LPS. (a) Venn map, (b) Rarefaction curve, (c) Shannon index curve, (d) Rank abundance curve. Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT).

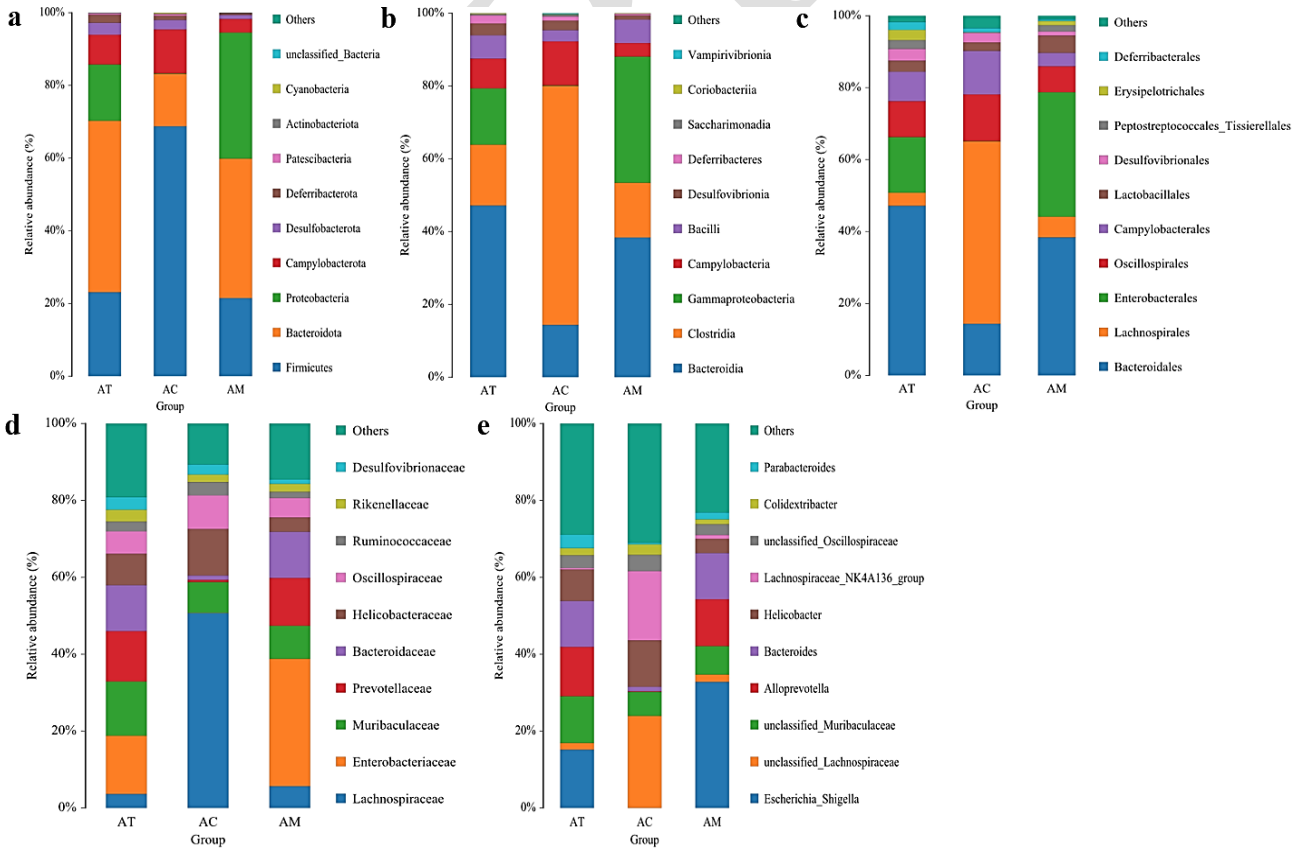

Fig. 5: The effect of MP on intestine microbiota in different taxa in mice induced by LPS. (a) Phylum, (b) Class, (c) Order, (d) Family, (e) Genera. Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT).

detected in group AM (38.37%, 34.62%, 7.21%) and AT (47.16%, 15.46%, and 9.99%), respectively (Fig. 5c). At the family level, *Lachnospiraceae* (50.69%), *Helicobacteraceae* (12.10%), and *Oscillospiraceae* (8.73%) were the primary families in group AC, *Enterobacteriaceae* (33.13%), *Prevotellaceae* (12.44%) and *Bacteroidaceae* (12.02%) were the dominating families in group AM, and *Enterobacteriaceae* (15.16%), *Muribaculaceae* (14.09%) and *Prevotellaceae* (13.11%) were the prominent families in group AT (Fig. 5d). At the genus level, unclassified *Lachnospiraceae* (23.91%), *Lachnospiraceae* NK4A136 group (18.00%), and *Helicobacter* (12.10%) were mainly examined in group AC, *Escherichia Shigella* (32.78%), *Alloprevotella* (12.19%), and *Bacteroides* (12.09%) were the staple genera in group AM. *Escherichia Shigella* (15.16%), *Alloprevotella* (12.84%) and unclassified *Muribaculaceae* (12.17%) were the primary genera in group AT (Fig. 5e). The species distribution bubble map showed *Firmicutes*, *Bacteroidota*, *Proteobacteria*, and *Campylobacterota* as the main phyla in ICR animals (Fig. 6a). The dominating genera in different groups were unclassified *Lachnospiraceae*, *Lachnospiraceae* NK4A136 group, and *Helicobacter* in group AC, *Escherichia*, *Shigella*, unclassified *Muribaculaceae*, *Alloprevotella*, *Bacteroides*, and *Helicobacter* (Fig. 6b).

Marker bacteria in the microbiota of LSP-inducing mice treated with MP: Beta diversity analysis revealed a shorter distance between group AC and AT compared to the distance between AC and AM (Fig. 7A-D). Further exploration of the marker bacteria among the three groups indicated a higher abundance of *Erysipelatoclostridiaceas* in group AC, *Proteobacteria*, *Enterobacteriaceae*, Enterobacterales, *Gammaproteobacteria*, *Escherichia*, *Shigella*, *Enterococcus*, and *Enterococcaceae* were higher in AM. At the same time, *Peptococcus*, ASF356, *Roseburia*, unclassified *Prevotellaceae*, *Acetatifactor*, *Vampirivibrionia*, and *Cyanobacteria* were found to be higher in AT (Fig. 8A, B). The ANOVA analysis indicated significant differences in bacterial phyla among the groups.

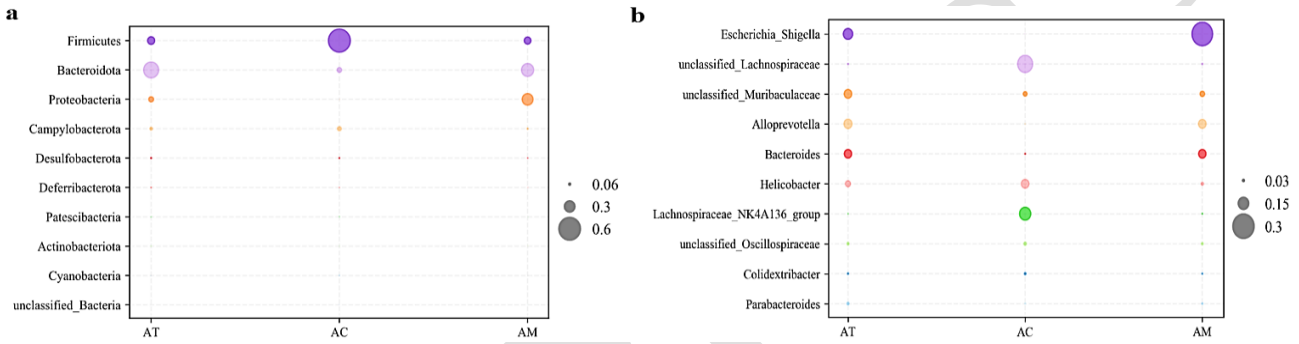

Fig. 6: The effect of MP on intestine microbiota in ICR mice in species distribution bubble map. (a) Phylum, (b) Genera. Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT).

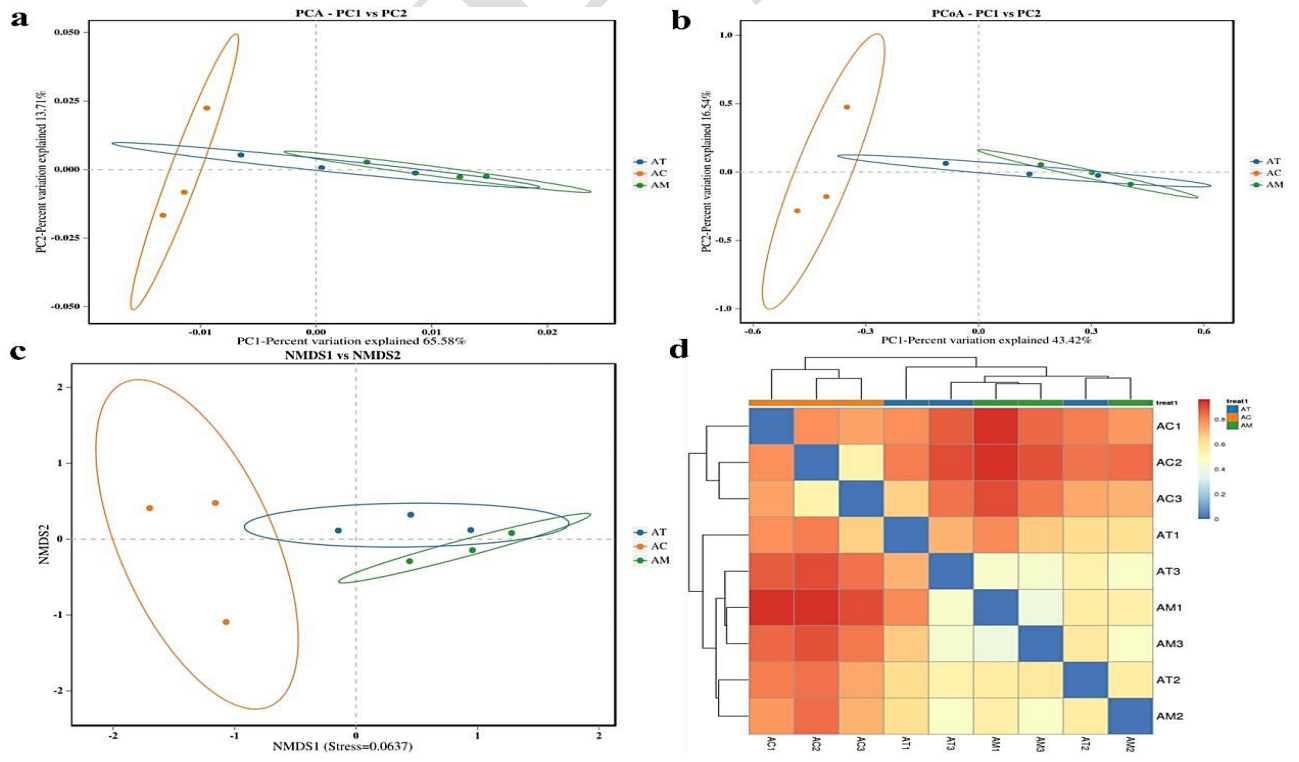

Fig. 7: Beta diversity analysis of ICR animals in different groups. The figure illustrates the shifts in microbial composition, with a significant increase in beneficial bacteria such as *Lactobacillus* in the MP-treated group, indicating improved gut health. (a) PCA, (b) PCoA, (c) NMDS, (d) Sample Clustering Heat Map.

Bacteroidota levels were significantly higher in group AT compared to AC ($P<0.05$), while *Proteobacteria* were markedly elevated in group AM relative to AC ($P<0.05$). *Firmicutes* abundance in group AC was substantially greater than in both AM ($P<0.01$) and AT ($P<0.01$). Similarly, *Cyanobacteria* levels in AC were significantly higher than those in AM ($P<0.05$) and AT (P<0.05) (Fig. 9A). At the genus level, multiple taxa exhibited notable reductions in group AM compared to AC. These included ASF356 (P<0.05), *Butyricicoccus* (P<0.05), *Candidatus Arthromitus* (P<0.05), Family XIII UCG 001 (P<0.001), *Harryflintia* (P<0.01), *Lachnospiraceae* NK4A136 group ($P<0.001$), *Peptococcus* (P<0.0001), *Roseburia* (P<0.05), *Tyzzerella* (P<0.01), UCG_005 ($P<0.05$), unclassified Clostridia vadinBB60 group (P<0.05), unclassified Lachnospiraceae ($P<0.01$), unclassified Peptococcaceae (P<0.01), unclassified Prevotellaceae (P<0.01), and unclassified RsE47 termite group (P<0.05). In contrast, *Escherichia-Shigella* was significantly enriched in AM (P<0.01) (Fig. 9B).

Group AT also showed reduced abundances of *Butyricicoccus* (P<0.05), *Candidatus* Arthromitus ($P<0.05$), Family XIII UCG 001 ($P<0.01$), Harryflintia (P<0.01), *Lachnospiraceae* NK4A136 group (P<0.001), *Peptococcus* (P<0.0001), *Roseburia* (P<0.05), *Tyzzerella* (P<0.01), UCG_005 (P<0.01), unclassified *Clostridia* vadinBB60 group (P<0.05), unclassified *Lachnospiraceae* (P<0.01), unclassified *Peptococcaceae* (P<0.01), unclassified *Prevotellaceae* (P<0.05), and unclassified RsE47 termite group (P<0.05) relative to AC. Conversely, *Erysipelatoclostridium* was significantly elevated in AT (P<0.05) (Fig. 9B).

Network analysis of the microbiota in ICR animals: The results of the network analysis identified key genera influencing the microbiota of ICR animals. Among the top 42 abundant genera, *Escherichia*, *Shigella*, unclassified *Muribaculaceae*, *Bacteroides*, *Alloprevotella*, *Lachnospiraceae* NK4A136 group, and unclassified

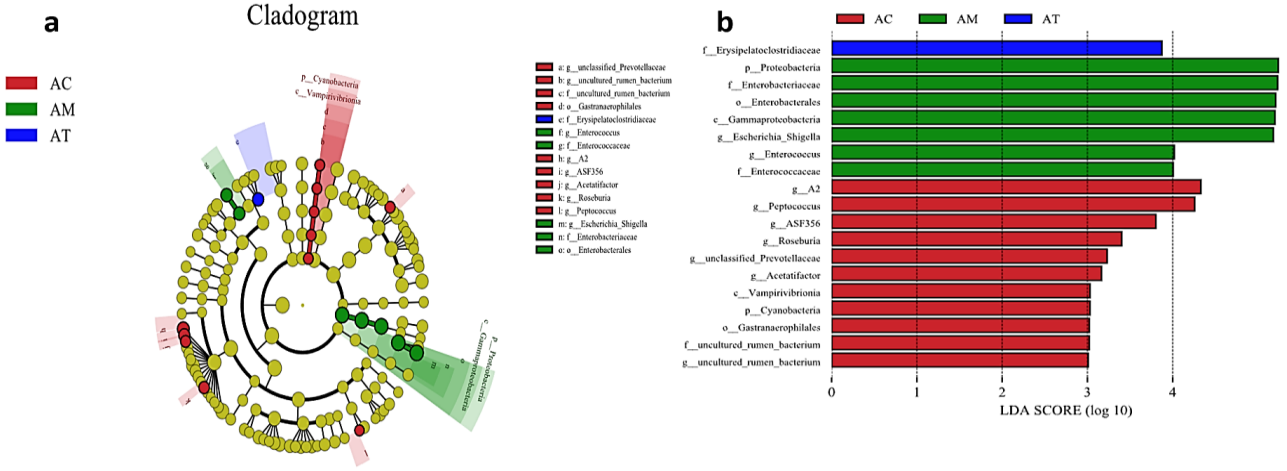

Fig. 8: Distinguished bacteria in mice in different groups (a) LEfSe analysis, (b) LDA score. Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT).

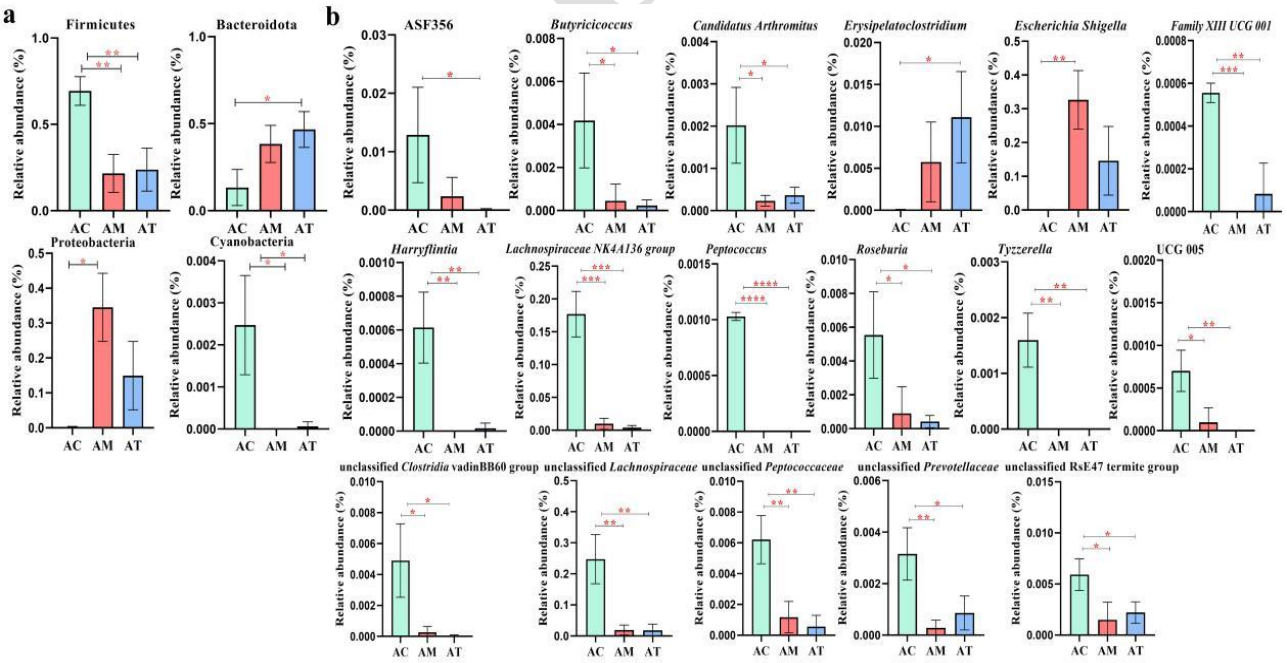

Fig. 9: Marker microbiota analysis of ICR animals in different groups via ANOVA. (a) Phylum, (b) Genera. Significance is presented as *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001; data are presented as the mean ± SEM (n=3). Control group (AC); LPS-induced group (AM); Morel Polysaccharides treatment group (AT).

Lachnospiraceae were identified as crucial genera affecting the microbiota composition in ICR animals (Fig. 10).

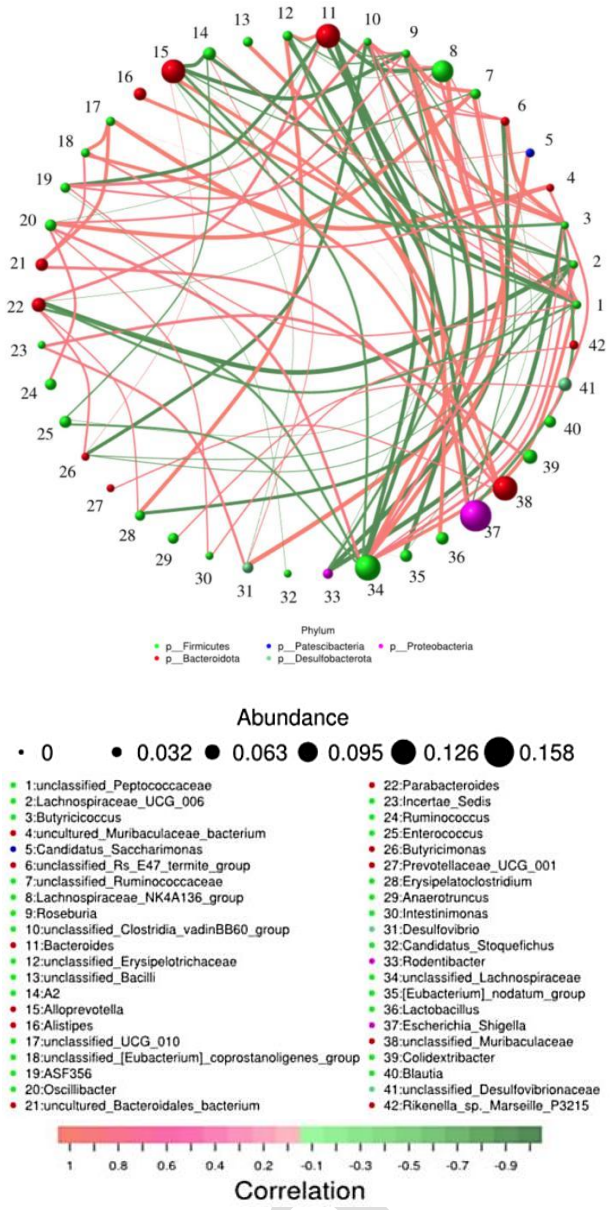

Fig. 10: Network analysis of microbiota in ICR animals.

DISCUSSION

Morel, a renowned traditional mushroom, has been reported to possess microbiota-modulating polysaccharides (Badshah *et al.,* 2021). Herein, we investigated the impact of Morel Polysaccharide (MP) on the gut flora of mice induced by Lipopolysaccharide (LPS). In agreement with previous research (Li *et al.,* 2020b; Iozon *et al.,* 2022; He *et al.,* 2023), LPS-induced intestinal villi damage, while mice treated with MP exhibited significantly higher average weights than LPS-induced animals ($P<0.05$). Although body weight loss did not change significantly within 24 hours of LPS treatment, extending the duration of the experiment could provide further insights into the long-term effects of MP on body weight and intestinal health. Pathological analysis further revealed that MP could alleviate intestinal damage by enhancing villus integrity, which is characterized by increased villus height and a higher ratio of villus height to crypt depth, as well as decreased crypt depth, in mice. These findings suggest that MP could mitigate weight loss by preserving intestinal integrity.

TNF-α is a pro-inflammatory cytokine (Young *et al.,* 2017; Cui *et al.,* 2023), and IL-10, an anti-inflammatory cytokine (Saraiva *et al.,* 2020; Abd-Elfatah *et al.,* 2023), play crucial roles in immune regulation. In line with the results of a previous experiment (Wang *et al.,* 2022), LPS induced higher levels of TNF-α ($P<0.05$) and lower IL-10 levels ($P<0.01$) in mice. However, mice treated with MP reduced TNF-α and increased IL-10 levels, indicating that MP could attenuate inflammatory responses. Nitric oxide, another inflammatory mediator, can contribute to septicemia when combined with other inflammatory factors (Hwang *et al.,* 2023). Interestingly, mice treated with MP exhibited significantly higher levels of NO ($P<0.05$). This suggests that MP could play a role in reducing inflammatory responses in animals. Enzymes like SOD, GSH-Px, and MDA are vital components of the antioxidant system (Hu *et al.,* 2023). In line with previous findings in female mice induced by LPS (He *et al.,* 2023), our findings showed higher MDA levels and lower SOD and GSH-Px levels in ICR animals. However, mice supplemented with MP demonstrated higher SOD and GSH-Px contents and lower MDA levels, indicating that MP could enhance antioxidant capacity in mice.

The microbiota plays a crucial role in host homeostasis, and intestinal flora disturbance is linked to various diseases (Heleno *et al.,* 2013; Ismael *et al.,* 2022). Microbiota analysis in this study, generating 821,405 raw data and 721,719 filtered data from mouse samples, resulted in 1843 Operational Taxonomic Units (OTUs), with 9.44% shared OTUs among different mouse groups. No significant difference was found in alpha diversity in mice (Table 2), consistent with previous results in female mice challenged with LPS and in liver-damaged mice (He *et al.,* 2023). In the current study, it appears that LPS induced intestinal dysbiosis, but the MP treatment partly restored the microbiota balance in mice. Firmicutes and Bacteroides, the main phyla in healthy hosts (Qiu *et al.,* 2022), showed abundances of 83.11%, 59.85%, and 70.26% in groups AC, AM, and AT, respectively, indicating that MP could regulate microbiota in animals. Morel polysaccharide treatment helped rebalance the F/B ratio, coinciding with reduced inflammation, improved antioxidant status, and restored villus structure, indicating its potential therapeutic role in restoring gut microbial homeostasis. It is speculated that an increase in beneficial bacteria in the gut could be associated with enhanced gut barrier function and reduced inflammation, contributing to the improved intestinal health observed in MP-treated mice. In general, LPS increased the abundance of *Escherichia* and *Shigella* (32.78%) in group AM, while MP decreased it in AT (15.16%). *Escherichia Shigella*, a significant diarrhea pathogen that breaks the intestinal mucosa (Eileen *et al.,* 2019), was inhibited by MP, suggesting a potential mitigation of gut damage. Network analysis further identified *Escherichia* and *Shigella* as key genera interacting with others. Further surveying showed that the relative abundance of four phyla and seventeen genera was significantly different among the three animal

teams. Notably, *Candidatus Arthromitus*, known for its protective effect on the intestine (Peng *et al.,* 2023), exhibited slightly higher abundance in MP-treated animals (0.00036%) compared to group AM (0.00023%). A previous study found a low abundance of Family XIII UCG 001 in residents with homocystinuria (Rizowy *et al.,* 2020), and MP-treated animals had a slightly higher abundance of Family XIII UCG 001 in AT (0.000083117%) than AM (0). Similarly, a lower abundance of unclassified *Prevotellaceae* and unclassified RsE47 termite group was observed in mice induced by LPS, consistent with findings in diabetic mice (Raheel *et al.,* 2022; Zhou *et al.,* 2023) and rats with rheumatoid arthritis (Li *et al.,* 2022). However, MP supplementation resulted in a higher abundance of these two genera. These observations collectively suggest that MP may contribute to restoring the balance of the intestinal microbiota in animals.

Morel-derived polysaccharides likely influence gut microbiota through several interconnected mechanisms. Firstly, these polysaccharides act as prebiotics, selectively promoting the growth of beneficial microbiota such as *Lactobacillus* and *Bifidobacterium* species (Zhao *et al.,* 2023). Secondly, they are fermented by gut bacteria into short-chain fatty acids (SCFAs) in the colon. These SCFAs not only support colonic health but also modulate pH and immune responses, indirectly shaping microbial community structure (Pi *et al.,* 2024). Secondly, Morel polysaccharides may alter microbial gene expression related to carbohydrate metabolism and quorum sensing, thereby influencing microbial composition and metabolic output. Additionally, emerging evidence suggests they may enhance mucosal barrier integrity and reduce inflammation, creating a favorable environment for commensal bacteria. Future studies utilizing metagenomic and metabolomic profiling could clarify these pathways and their role in host-microbe interactions (Zhao *et al*., 2023; Pi *et al.,* 2024). The sample size for microbiota analysis (n=3 per group) is a limitation of the present study, which has less variation and diversity among the microbiota.

Conclusions: Our study has unveiled the potential of Morel polysaccharide (MP) in ameliorating intestinal damage induced by LPS through the regulation of oxidation resistance, inflammatory factors, and microbiota. Our findings suggest that MP could be a promising candidate for developing tailored therapeutic interventions targeting gut health. Further research into the mechanistic underpinnings and clinical applications of MP will be pivotal in unlocking its full potential for addressing intestinal pathologies and advancing the field of gut health therapeutics.

Data availability statement: All raw data from ICR animals were deposited in the NCBI Sequence Read Archive under accession number: PRJNA1066208.

Ethics statement: All the experiment procedures were guided by the instructions and approval of the ethics committee of Yunnan Agricultural University (SQ202503087).

Authors' contribution: YY, LL, and YS: research idea and methodology. YY, LL, QL, and JL: reagents, materials, and analysis tools. YY, LL, and YS: writing – original draft and preparation. YY and YS: writing, review, and editing. YS: visualization and supervision. YS: Funding and supervision. All authors have known and approved the final manuscript.

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REFERENCES

Abd-Elfatah EB, Fawzi EM, Elsheikh HA, *et al.,* 2023. Prevalence, virulence genes and antibiogram susceptibility pattern of staphylococcus aureus and streptococcus agalactiae isolated from mastitic ewes. Int J Vet Sci 12(2):152-160.

Ahmad S, Yousaf S, Tahir K, *et al*., 2023. Effects of co-supplementation of β-galacto-oligosaccharides and methionine on breast meat quality, meat oxidative stability and selected meat quality genes in broilers. Pak Vet J 43(3): 428-434.

Anwar U, Yousaf M, Mirza A, *et al.,* 2023. Impact of stored wheat-based feed on gut morphology, digesta viscosity and blood metabolites of broiler chickens. Pak Vet J 43(1): 179-183.

Badshah SL, Riaz A, Muhammad A, *et al*., (2021) Isolation, Characterization, and Medicinal Potential of Polysaccharides of Morchella esculenta. Molecules 26 (5): 1459.

Bolger AM, Lohse M and Usadel B, 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15): 2114-20.

Chai X, Wang J, Li HP, *et al*., 2023. Intratumor microbiome features reveal antitumor potentials of intrahepatic cholangiocarcinoma. Gut Microbes 15(1): 2156255.

Cui W, Liu LL, Du X, *et al*., 2023. Rutaecarpine regulates the expression of pro-inflammatory cytokines to induce protective effects in the murine model of acute reflux esophagitis. Pak Vet J 43(3): 545-552.

Edgar RC, Haas BJ, Clemente JC, *et al*., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16): 2194-2200.

Eileen MB and Myron ML, 2019. A tale of two bacterial enteropathogens and one multivalent vaccine. Cell Microbiol 21(11): e13067.

El-Sayed A, Aleya L and Kamel M, 2021. Microbiota's role in health and diseases. Environ Sci Pollut R 28(28): 36967-36983.

Fan X, Jin Y, Chen G, *et al*., 2021. Gut microbiota dysbiosis drives the development of colorectal cancer. Digestion 102(4): 508-515.

Fehily SR, Basnayake C, Wright EK and Kamm MA, 2021. The gut microbiota and gut disease. Intern Med J 51(10): 1594-1604.

Ferreira R, Heleno A, Reis S, *et al*., 2021. The higher antioxidant activity of polysaccharides isolated from Morchella sp. mushrooms under different drying conditions: A process optimization study. Int J Biol Macromol 166: 711-719.

Fu YJ, Xu B, Huang SW, *et al*., 2021. Baicalin prevents LPS-induced activation of TLR4/NF-kappaB p65 pathway and inflammation in mice via inhibiting the expression of CD14. Acta Pharmacol Sin 42(1): 88-96.

Gao N, Chen C, Ji Y, *et al*., 2023. Multi-locus sequence typing of *Streptococcus equi* subspecies *zooepidemicus* strains isolated from donkeys: A novel genotype associated with donkey infectious endometritis. Pak Vet J 43(4): 838-841.

He Y, Wang D, Liu K, *et al.* 2023 Sodium humate alleviates LPS-induced intestinal barrier injury by improving intestinal immune function and regulating gut microbiota. Mol Immunol 161: 61-73.

Heleno SA, Stojković D, Barros L, *et al*., 2013. A comparative study of chemical composition, antioxidant and antimicrobial properties of Morchella esculenta (L.) Pers. from Portugal and Serbia. Food Res Int 51(1): 236-243.

Hu SW, Wang P, Ke JH, *et al*., 2023. Protective effect of Peucedanum praeruptorum Dunn extract on oxidative damage of LLC PK1 cells induced by H2O2. Exp Ther Med 26(5): 517.

Huang D, Zhang M, Chen W, *et al*., 2018. Structural elucidation and osteogenic activities of two novel heteropolysaccharides obtained from water extraction residues of Cibotium barometz. Ind Crop Prod 121: 216-225.

Huo W, Qi P, Cui LJ, *et al*., 2020. Polysaccharide from wild morels alters the spatial structure of gut microbiota and the production of short-chain fatty acids in mice. Biosci Microbiota Food Health, 39(4): 219-226.

Hwang S, Wang J, Lee J, *et al*., 2023. Ginseng Sprouts Attenuate Mortality and Systemic Inflammation by Modulating TLR4/NF-κB Signaling in an LPS-Induced Mouse Model of Sepsis. Int J Mol Sci 24(2): 1583.

Iozon I, Cernea M, Buza V, *et al*., 2022. Inulin effect on intestinal mucus-secreting cells. Pak Vet J 43(3): 435-441.

Ismael E, Ismail E, Khalefa H, *et al*., 2022. Evaluation of *Saccharomyces cerevisiae* yeast fermentate and xylanase in reduced energy diet fed to broiler chicken. Int J Vet Sci 11(2): 141-150.

Jayachandran M, Xiao J and Xu B, 2019. A critical review on health promoting benefits of edible mushrooms through gut microbiota. Int J Mol Sci 20(10): 2560.

Jia X, Xu W, Zhang L, *et al*., 2021. Impact of Gut Microbiota and Microbiota-Related Metabolites on Hyperlipidemia. Front Cell Infect Microbiol 11: 634780.

Kirn N, Lu J, Aqib I, *et al*., 2023. Annotating susceptibility potential of single, double, tri and tetra mixed infection bacteria against non-beta lactam antibiotics. Pak Vet J 43(3): 596-600.

Li C, Ma D, Zhou H, *et al*., 2020a. Effects of different doses lipopolysaccharides on the mucosal barrier in mouse intestine. Res Vet Sci 133: 75-84.

Li RX, Li J, Zhang SY, *et. al*., 2020b. Attenuating effect of melatonin on lipopolysaccharide-induced chicken small intestine inflammation. Poult Sci 97(7): 2295-2302.

Li Y, Liu C, Luo J, *et al*., 2022. Ershiwuwei Lvxue Pill alleviates rheumatoid arthritis by different pathways and produces changes in the gut microbiota. Phytomedicine 107: 154462.

Li Y, Xia S, Jiang X, *et al*., 2021. Gut Microbiota and Diarrhea: An Updated Review. Front Cell Infect Microbiol 11: 625210.

Lima J, Manning T, Rutherford KM, *et al*., 2021. Taxonomic annotation of 16S rRNA sequences of pig intestinal samples using MG-RAST and QIIME2 generated different microbiota compositions. J Microbiol Meth 186: 106235.

Maki KA, Wolff B, Varuzza L, *et al*., 2023. Multi-amplicon microbiome data analysis pipelines for mixed orientation sequences using QIIME2: Assessing reference database, variable region and pre-processing bias in classification of mock bacterial community samples. PLoS One 18(1): e0280293.

Meng X, Zhang G, Cao H, *et al*., 2020. Gut dysbacteriosis and intestinal disease: mechanism and treatment. J Appl Microbiol 129(4): 787-805.

Nawaz Z, Rasool MH, Siddique AB, *et al*., 2020. Frequency and molecular detection of *Giardia intestinalis* in children attending pediatrics of Punjab, Pakistan. Jundish J Microbiol 13(1): 1-6.

Peng X, Yi X, Deng N, *et al*., 2023. Zhishi Daozhi decoction alleviates constipation induced by a high-fat and high-protein diet via regulating intestinal mucosal microbiota and oxidative stress. Front Microbiol 14: 1214577.

Pi Y, Fang M, Li Y, *et al.,* 2024. Interactions between Gut Microbiota and Natural Bioactive Polysaccharides in Metabolic Diseases: Review. Nutrients 16(17): 2838.

Qiu P, Ishimoto T, Fu L, *et al*., 2022. The Gut Microbiota in Inflammatory Bowel Disease. Front Cell Infect Mi 12: 733992.

Raheel I, Orabi A, Erfan A, *et al*., 2022. Intestinal tract of broiler chickens as a reservoir of potentially pathogenic curli producing esβl *Escherichia coli*. Int J Vet Sci 11(4): 498-503.

Ren YY, Zhaxi M, Liu A, *et al*., 2023. Revealing the fungi microbiome difference of suffolk cross with Tibetan sheep on plateau, Pak Vet J 43(4): 748-756.

Rizowy GM, Poloni S, Colonetti K, *et al*., 2020. Is the gut microbiota dysbiotic in patients with classical homocystinuria? Biochimie 173: 3-11.

Rognes T, Flouri T, Nichols B, *et al*., 2016. VSEARCH: a versatile open-source tool for metagenomics. PeerJ, 4: e2584.

Saraiva M, Vieira P and O'Garra A, 2020. Biology and therapeutic potential of interleukin-10. J Exp Med 217(1): e20190418.

Tang C, Sun J, Zhou B, *et al*., 2018. Effects of polysaccharides from purple sweet potatoes on immune response and gut microbiota composition in normal and cyclophosphamide treated mice. Food Funct 9(2): 937-95.

Tang Y, Chen J, Li F, *et al*., 2019. Antioxidant and Antiproliferative Activities of Modified Polysaccharides Originally Isolated from Morchella Angusticepes Peck. J Food Sci 84(3): 448-456.

Thursby E and Juge N, 2017. Introduction to the human gut microbiota. Biochem J 474(11): 1823-1836.

Wang W, Xue C and Mao X, 2020. Radioprotective effects and mechanisms of animal, plant and microbial polysaccharides. Int J Biol Macromol 153: 373-384.

Wang WB, Li JT, Hui Y, *et al*., 2022. Combination of pseudoephedrine and emodin ameliorates LPS-induced acute lung injury by regulating macrophage M1/M2 polarization through the VIP/cAMP/PKA pathway. Chin Med 17(1): 19.

Wassenaar TM and Zimmermann K, 2018. Lipopolysaccharides in food, food supplements, and probiotics: should we be worried? Eur J Micro Immu 8(3): 63-69.

Wen Y, Peng D, Li C, *et al*., 2019. A new polysaccharide isolated from *Morchella importuna* fruiting bodies and its immunoregulatory mechanism. Int J Biol Macromol 137: 8-19.

Xiong C, Li P, Luo Q, *et al*., 2021. Induction of apoptosis in HeLa cells by a novel peptide from fruiting bodies of *Morchella importuna* via the mitochondrial apoptotic pathway. Evid Based Compl Alternat Med. 2021: 5563367.

Xu Y, Tang J, Wang Y, *et al*., 2022. Large-scale commercial cultivation of morels: current state and perspectives. Appl Microbiol Biot 106(12): 4401-4412.

Young RS, Wiles BM, Mcgee DW. (2017). IL-22 enhances TNF-α- and IL-1-induced CXCL8 responses by intestinal epithelial cell lines. Inflammation 40(5): 1726-1734.

Yu X, Jiang W, Kosik RO, *et al*., 2022. Gut microbiota changes and its potential relations with thyroid carcinoma. J Adv Res 35: 61-70.

Zafar H, Rahman SU, Ali S and Javed MT, 2019. Evaluation of a *Salmonella* strain isolated from honeybee gut as a potential live oral vaccine against lethal infection of *Salmonella* Typhimurium. Pol J Micro 68(2): 173-183.

Zhao Q, Jiang Y, Zhao Q, *et al.,* 2023. The benefits of edible mushroom polysaccharides for health and their influence on gut microbiota: a review. Front Nutr 10: 1213010.

Zhou R, He D, Zhang H, *et al.,* 2023. Ginsenoside Rb1 protects against diabetes-associated metabolic disorders in Kkay mice by reshaping gut microbiota and fecal metabolic profiles. J Ethnopharmacol 303: 115997.

Zhou XH, Zezi MYA, Li DD and Wang J, 2022. Telmisartan ameliorates LPS-induced pneumonia in rats through regulation of the PPARγ/NF-κB pathway. Microbiol. Immunol 66(7): 371-378.