

Article

Liposomal Delivery of *Macleaya cordata* Extract Alleviates Bacterial Diarrhea Through Intestinal Barrier Restoration, Microbiota Remodeling, and Inhibition of Inflammatory Factor Release

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Abstract

Background/Objectives: To overcome bottlenecks in the application of *Macleaya cordata* extract (MCE) in veterinary traditional Chinese medicine, such as low bioavailability of its active ingredients, gastrointestinal irritation, and muscular toxicity, this study aimed to develop a liposomal nano-delivery system loaded with MCE (MCE-Lips) to achieve the core objective of “enhancing efficacy and reducing toxicity” and to explore its potential application and mechanism of action in treating bacterial diarrhea. **Methods:** MCE-Lips were prepared using the thin-film dispersion method, and their physicochemical properties—particle size, encapsulation efficiency, and drug loading capacity—were characterized. In vitro, cytotoxicity against skeletal muscle cells and NCM460 intestinal epithelial cells was evaluated using the CCK-8 assay. The release of lactate dehydrogenase (LDH) from skeletal muscle cells was measured with an LDH assay kit. The expression levels of inflammatory factors (TNF- α , IL-6, and IL-1 β) in both cell types were determined through ELISA. A fluorescent probe was employed to assess cell membrane integrity. The effect of MCE-Lips on the expression of tight junction proteins (ZO-1, Occludin, and Claudin-5) was evaluated via immunofluorescence. Acute toxicity was examined through H&E staining. A bacterial diarrhea model was established using *Escherichia coli* in mice, and comprehensive safety and efficacy were assessed through hematological tests and gastrointestinal motility evaluation. Finally, untargeted metabolomics and 16S rRNA sequencing were utilized to investigate the underlying mechanisms of action. **Results:** The prepared MCE-Lips had an average particle size of 86.49 nm and a high encapsulation efficiency of 89.07%. In vitro experiments demonstrated that MCE-Lips significantly alleviated skeletal muscle cell damage, reduced LDH release ($p < 0.05$), and effectively inhibited the expression of inflammatory factors IL-6, TNF- α , and IL-1 β ($p < 0.05$). In NCM460 cells, MCE-Lips exhibited a more pronounced inhibitory effect on LPS-induced release of TNF- α ($p < 0.01$), IL-6 ($p < 0.0001$), and IL-1 β ($p < 0.0001$) and enhanced intestinal barrier function by upregulating the expression of tight junction proteins ZO-1 ($p < 0.001$), Occludin ($p < 0.01$), and Claudin-5 ($p < 0.01$). In the bacterial diarrhea model, MCE-Lips showed excellent anti-diarrheal efficacy ($p < 0.01$). Hematological analysis indicated no systemic toxicity. At the endocrine level, the high-dose group significantly reduced motilin (MTL) levels ($p < 0.01$), which slowed intestinal motility and prolonged chyme retention, thereby alleviating diarrhea



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symptoms. Mechanistic studies revealed that it acts by regulating the intestinal metabolic profile and microbial community structure, with *Desulfovibrio*, *Enterococcus*, and *Streptococcus* identified as key characteristic differential genera. **Conclusions:** For the first time, an MCE liposome nanoparticle system was constructed, and untargeted metabolomics combined with 16S rRNA sequencing were employed to elucidate its anti-diarrheal mechanism. MCE-Lips exerts excellent antibacterial diarrhea effects through multiple mechanisms, including direct cytoprotection and anti-inflammatory action, enhancement of the intestinal barrier, regulation of gut function, and remodeling of the gut microecology. This work provides a novel paradigm for plant-derived nano-anti-diarrheal agents. The systematic evaluation of the pharmacodynamics of MCE-Lips in a piglet bacterial diarrhea model will lay a solid foundation for its eventual market application.

Keywords: liposomes; pharmacokinetics; anti-diarrheal; intestinal microbiota; tight junction proteins; metabolomics

1. Introduction

The antibiotic resistance crisis resulting from antibiotic misuse poses a significant threat to global public health security. To address this challenge, China implemented a comprehensive “antibiotic ban” in animal feed in 2020, driving an annual growth rate of 18.7% in the plant-derived feed additives market [1]. MCE, derived from the Papaveraceae plant *Macleaya cordata* [2], serves as a representative antibiotic-alternative phytomedicine. Its main benzophenanthridine alkaloids, Sanguinarine (SA) and Chelerythrine (CHE), exhibit significant antibacterial, anti-inflammatory, and growth-promoting activities [3–6] and are commonly used in livestock and poultry industries [7,8]. Based on its distinctive safety profile and chemical composition, MCE has been officially included in the EU Catalogue of Feed Materials and approved as a safe source of nutritional additives for animals. Significant progress has also been achieved in the industrial application of MCE. For instance, Camas Inc. in the United States has developed a botanical bactericide named QWEL, with SA as its core active ingredient. In the field of anti-inflammatory applications, two second-class veterinary traditional Chinese medicines—“Boluohui san” and “Bopu Zongjian”—have been developed. These innovative products have been approved by the Ministry of Agriculture and Rural Affairs of China, representing a breakthrough in antibiotic alternatives. However, the low oral bioavailability [9,10], chemical instability, and dose-dependent hepatorenal toxicity of SA and CHE severely limit their clinical application [11,12]. Previous studies have confirmed that free MCE causes significant local irritation upon intramuscular injection, and over 90% of the parent drug is excreted via the intestine, failing to achieve effective plasma concentrations [13,14].

The intestine, the largest immune organ, plays a crucial role in human health, with its microbial communities exerting significant influence [15]. Studies have demonstrated that MCE can beneficially modulate the gut microbiota by enhancing intestinal barrier function and antioxidant capacity, improving the growth performance and intestinal health of American eels [16]. Furthermore, MCE was found to regulate changes in intestinal microbiota metabolic pathways induced by heat stress [17]. Liu et al. investigated the effects of isoquinoline alkaloids from MCE on alleviating lipopolysaccharide (LPS)-induced intestinal epithelial damage in broilers. The results showed that isoquinoline alkaloids could mitigate LPS-induced intestinal epithelial injury and the disruption of gut homeostasis while enhancing anti-inflammatory and antioxidant capacities in broilers [18]. Zhang et al. explored how MCE improves immune responses and egg quality by enhancing

intestinal health and modulating the gut microbiota [19]. Furthermore, MCE demonstrated potential as an antibiotic alternative by improving gastrointestinal epithelial integrity and enhancing humoral immune responses in goats [20]. However, the complex mechanisms underlying MCE's regulation of intestinal metabolism remain unclear, making it difficult to precisely understand the interactions between the drug, gut microecology, and host health. Moreover, no prior studies have applied liposomes to the comprehensive “efficacy enhancement and toxicity reduction” development of MCE, and a deep analysis of its intestinal metabolic regulatory mechanisms is lacking.

Nanodelivery systems offer new strategies to overcome these bottlenecks. Liposomes, with their phospholipid bilayer structure [21], can encapsulate both hydrophilic and hydrophobic drugs, enhancing bioavailability by improving intestinal permeability, bypassing first-pass metabolism, and enabling targeted delivery [22,23]. This makes them ideally suited for the co-delivery of MCE's complex components. Studies have confirmed that liposomes can reduce the muscular toxicity of SA and prolong its systemic circulation time [24]. The long-circulating and targeting properties of liposomes could further promote the distribution and absorption of MCE in vivo. Intramuscular (IM) injection is an ideal administration route for liposomes [25]. Research has demonstrated that liposome-encapsulated drugs like paclitaxel [26,27] and doxorubicin [28,29] significantly enhance efficacy and reduce toxicity when administered via injection, providing important references for MCE formulation innovation. Constructing MCE-Lips can significantly improve the solubility and stability of MCE, reduce irritation, and thereby enhance its intramuscular bioavailability.

This study is the first to construct a liposomal nano-system loaded with MCE (MCE-Lips). By systematically evaluating its pharmacokinetic characteristics, acute toxicity, and anti-diarrheal efficacy, combined with non-targeted metabolomics and in vitro barrier repair experiments, we elucidated the synergistic mechanism of MCE-Lips through the regulation of organic acid metabolism, inhibition of inflammation, and enhancement of intestinal mucosal barrier function. This research provides theoretical and technical support for the development of nano-formulations of plant-derived antibiotic alternatives and offers evidence for the therapeutic targets and pathways of MCE in treating diarrhea and other diseases.

2. Materials and Methods

2.1. Materials

Macleaya cordata extract was prepared in-house by Hunan Agricultural University (Changsha, China). Sanguinarine, chelerythrine, dihydrosanguinarine, and dihydrochelerythrine (purity $\geq 98\%$) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Methanol and acetonitrile (both HPLC grade) were supplied by Spectrum Chemical Mfg. Corp. (Shanghai, China). Phosphoric acid was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydrochloric acid was provided by Hunan Huihong Reagent Co., Ltd. (Changsha, China). Sodium dihydrogen phosphate, disodium hydrogen phosphate, cholesterol, and soybean lecithin were acquired from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China).

2.2. Cell Lines and Culture

Human primary skeletal muscle cells (iCELL, HUM-iCell-s008, Shanghai, China) were cultured in medium containing 88% primary skeletal muscle cell basal medium (iCELL, iCell-s008-002h, Shanghai, China), 1% fetal bovine serum (FBS, AFD050, Abcell, Beijing, China), and 1% penicillin/streptomycin solution (SC118-01, seven, Beijing, China). Human normal colon epithelial cells (iCELL, NCM460, Shanghai, China) were cultured in medium containing 89% RPMI 1640 medium (C11875500BT, Gibco, Shanghai, China), 10% FBS

(AFD050, Abcell, Beijing, China), and 1% penicillin/streptomycin solution (SC118-01, seven, Beijing, China). They were maintained in a humid incubator at 37 °C with 5% CO₂.

2.3. Animals

ICR mice, half male and half female ($n = 80$, weight 18.0–22.0 g), were provided by Hunan Silaike Jingda Experimental Animal Co., Ltd. (Changsha, China). Animal studies were reviewed and approved by the Experimental Animal Ethics Committee of the Hunan University of Chinese Medicine (HNUCM11-2503-050) and complied with all institutional and national guidelines. The experimental animals were housed at the Hunan University of Chinese Medicine (license number: SYXK 2024-0019; approval date: 11 February 2025) under a temperature of 20–26 °C, humidity of 40–70%, and a 12 h light/12 h dark cycle.

2.4. Preparation of MCE-Lips

Accurately weigh 8 mg of MCE, 80 mg of soybean lecithin, and 13.33 mg of cholesterol and dissolve them in a chloroform–methanol (4:1, v/v) solution. Evaporate the solvent through rotary evaporation at 50 °C until a thin lipid film forms. Add phosphate-buffered saline (PBS) at pH 6.4, and hydrate the mixture at 50 °C for 1 h. The resulting dispersion is then ultrasonicated for 20 min using a probe ultrasonic apparatus in an ice bath (2 s ultrasonic pulses followed by 2 s pauses). Finally, filter the dispersion through 0.45 µm and 0.22 µm microporous membranes sequentially to obtain MCE-Lips.

2.5. Preparation of MCE-Lips Lyophilized Powder

Based on prior single-factor investigations, the final lyophilization protocol was established. The MCE-Lips were converted into a lyophilized powder using freeze-drying. A mixture of mannitol and glucose (25% w/v total, at a ratio of 3:2) was selected as the cryoprotectant. The cryoprotectants were added using a combination of internal and external loading methods, with the internal addition accounting for 40% of the total. The solution was pre-frozen in the freeze-dryer chamber at −55 °C for 6 h, followed by primary drying for 36 h to obtain the final lyophilized powder.

2.6. Characterization of MCE-Lips and Lyophilized Powder

The mean particle size, polydispersity index (PDI), and zeta potential of the liposomal formulations were determined using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK). All samples were appropriately diluted with PBS before each measurement. The morphology of the formulations was observed and photographed using transmission electron microscopy (TEM). The encapsulation efficiency (EE) and drug loading (DL) capacity were quantified via high-performance liquid chromatography (HPLC). The storage stability of both the MCE-Lips and the freeze-dried formulation was assessed. The encapsulation efficiency (EE%), defined as the percentage of encapsulated MCE ($W_2 - W_1$) relative to the total amount of drug used in the preparation (W_1), was calculated using the following formula:

$$EE(\%) = \frac{W_2 - W_1}{W_1} \times 100\% \quad (1)$$

Drug loading (DL), defined as the ratio of the amount of encapsulated drug (W_{Drug}) to the net weight of the lyophilized liposomes (W_{Lips}), was calculated as follows:

$$DL(\%) = \frac{W_{\text{Drug}}}{W_{\text{LIPS}}} \times 100\% \quad (2)$$

2.7. Cytotoxicity Assay

Skeletal muscle cells and intestinal epithelial NCM460 cells were seeded into 96-well plates at 1×10^4 cells per well. Drug-treated groups were exposed to media containing different drug concentrations (0.625, 1.25, 2.5, 5, 10 $\mu\text{g/mL}$). After 24 h of culture, 10 μL of CCK8 solution (SC119-02, seven, Beijing, China) was added to each well. Plates were incubated for an additional 2 h, and absorbance was measured at 450 nm using a microplate reader (Thermo Scientific, Waltham, MA, USA). Cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Control}} - A_{\text{Blank}}} \times 100\% \quad (3)$$

2.8. LDH Release Assay in Skeletal Muscle Cells

LDH release from skeletal muscle cells was detected using an LDH assay kit (ml098824, Enzyme-linked Biotechnology, Shanghai, China). Cells were seeded in 96-well plates at 1×10^4 cells per well. Drug-treated groups received media containing drugs at a 1.25 $\mu\text{g/mL}$ concentration and were cultured for 24 h. LDH release reagent was added to each well and incubated at 37 °C for 10 min. Subsequently, 60 μL of LDH detection working solution was added, and plates were incubated at room temperature in the dark for 30 min. Absorbance was measured at 490 nm, and LDH release was calculated.

2.9. ELISA Detection of Inflammatory Cytokines in Skeletal Muscle Cells

Skeletal muscle cells were seeded in 96-well plates at 1×10^4 cells per well. Drug-treated groups received drug-containing medium at a 1.25 $\mu\text{g/mL}$ concentration and were cultured for 24 h. Supernatants were collected, and levels of inflammatory cytokines IL-1 β (EHC002b, Neobioscience, Shenzhen, China), TNF- α (EHC103a, Neobioscience, China), and IL-6 (EHC007, Neobioscience, China) were determined according to ELISA. The experimental procedure was conducted as follows: after retrieving the required strips, dilution buffer, samples, or standards were added respectively and incubated at 37 °C in the dark for 90 min, followed by five washes; then, biotinylated antibody working solution was added and incubated at 37 °C in the dark for 60 min, followed by another five washes; next, enzyme conjugate working solution was added and incubated at 37 °C in the dark for 30 min, followed by five washes; thereafter, TMB substrate was added to each well and incubated at 37 °C in the dark for 15 min for color development; finally, stop solution was added, and, after mixing, the OD450 was measured within 3 min.

2.10. Fluorescent Probe Detection of Skeletal Muscle Cell Membrane Rupture

Skeletal muscle cells were seeded in culture dishes at 1×10^4 cells per well and cultured for 24 h. Drug-treated groups received drug-containing medium at 1.25 $\mu\text{g/mL}$ concentration and were cultured for an additional 24 h. Supernatant was discarded and cells were washed three times with PBS and then incubated with 0.1% ethidium bromide (EtBr, HY-D0021, MCE, Shanghai, China) at room temperature in the dark for 15 min. EtBr was discarded, cells were washed with PBS, and fluorescence was observed.

2.11. Cytotoxicity Assay in NCM460 Cells After LPS Stimulation

NCM460 cells were seeded in 96-well plates at 1×10^4 cells per well and cultured for 24 h. The model group received 100 μL of LPS (L2880, Sigma, Shanghai, China) at a final concentration of 1 $\mu\text{g/mL}$. Drug-treated groups received drug-containing medium at 0.63 $\mu\text{g/mL}$. After incubating for 2 h, LPS was added to the drug-treated groups, and the culture continued for 24 h. Then, 10 μL of CCK8 solution (SC119-02, seven, Beijing, China) was added to each well, and absorbance was measured at 450 nm.

2.12. Effect of Drugs on LPS-Induced Extracellular Release of Inflammatory Factors in NCM460 Cells

NCM460 cells were seeded in 96-well plates at 1×10^5 cells per well and cultured for 24 h. The LPS treatment group received 100 μ L of LPS (final concentration 1 μ g/mL). Drug-treated groups were incubated with 100 μ L of drug solution (0.63 μ g/mL) for 2 h, followed by LPS addition to achieve a final concentration of 1 μ g/mL. After 24 h of incubation, supernatants were collected, and levels of IL-6, TNF- α , and IL-1 β in NCM460 cell supernatants were measured through ELISA.

2.13. Immunofluorescence Detection of Drug Effects on ZO-1, Occludin, and Claudin-5 Protein Expression

NCM460 cells were seeded in 24-well plates (1×10^5 cells/well) and cultured for 24 h. The model group received LPS (final concentration of 1 μ g/mL). Drug-treated groups were pre-treated with the drug (0.63 μ g/mL) for 2 h before adding LPS, followed by 24 h of culture. Cells were fixed with 4% paraformaldehyde at room temperature for 20 min, washed three times with PBS, and blocked with 3% BSA at room temperature for 1 h. Primary antibodies against ZO-1 (Proteintech, 82870-7-RR, Wuhan, China), Occludin (Proteintech, 27260-1-AP, China), and Claudin-5 (Proteintech, 29767-1-AP, China) were added (300 μ L) and incubated overnight at 4 $^{\circ}$ C. After washing three times with PBS (5 min each), fluorescent secondary antibodies were added and incubated at 37 $^{\circ}$ C in the dark for 1 h. Cells were washed again with PBS three times, mounted with DAPI, and observed/photographed under a fluorescence microscope.

2.14. Safety Assessment

To evaluate the clinical safety of MCE-Lips, acute toxicity tests were conducted on ICR mice. The MCE-Lips group received an intramuscular injection at a volume of 10 mL/kg (76.85 mg/kg). The blank control group received an equivalent volume of 0.9% saline (C23070703, Zhejiang Guojing Pharmaceutical Co., Ltd., Lishui, China). Behavioral responses, body weight changes, mortality, and histopathological changes in major organs (heart, liver, spleen, lung, kidney) stained with hematoxylin and eosin (HE) were observed in mice for 14 days after intramuscular injection of the maximum dose of MCE-Lips.

2.15. In Vivo Pharmacodynamics (Anti-Diarrheal Effect)

To investigate the anti-diarrheal effect of MCE-Lips, 60 ICR mice were randomly divided into 6 groups ($n = 10$ /group, half male and half female). The bacterial diarrhea model was established through intraperitoneal injection of 0.6 mL/mouse of an E. coli suspension (4×10^{10} CFU/mL). The blank group received an equivalent volume of 0.9% saline. The anti-diarrheal effect of MCE-Lips was evaluated. Diarrhea latent time, diarrhea incidence, and diarrhea index (calculated based on loose stool rate and grade) were observed and recorded to quantify anti-diarrheal activity. Grouping and dose design are detailed in Table 1.

Table 1. Trial grouping and dose design.

Group	Mouse	
	Concentration (mg/mL)	Dose (mg/kg)
Blank group	/	/
Model group	/	/
Chuanxinlian injection	1 g/mL	5 g/kg
Low MCE-Lips	466.8 mg/mL	6.3 mg/kg
Medium MCE-Lips	886.5 mg/mL	12.5 mg/kg
High MCE-Lips	1773.0 mg/mL	25.0 mg/kg

2.16. Hematology and Inflammatory Factor Level Detection

To preliminarily elucidate the potential mechanism of MCE-Lips against diarrhea, orbital venous blood was collected from mice in the above diarrhea model experiment. Automated blood cell analysis was performed to assess immune status. Serum levels of key inflammatory factors (IL-6, TNF- α , CRP) and gastrointestinal hormones (MTL, GAS) were measured using ELISA.

2.17. Non-Targeted Metabolomics

To further investigate the regulatory effect of MCE-Lips on the intestinal microenvironment, intestinal contents from each group of mice were collected and sent to Biomarker Technologies Co., Ltd. for non-targeted metabolomics analysis. Metabolic profiles were detected using UPLC-QTOF-MS technology. Differential metabolites were screened using PCA and volcano plots. Key metabolic pathways regulated by MCE-Lips were revealed through KEGG pathway enrichment analysis.

2.18. 16S rRNA Gene Sequencing Analysis of Gut Microbiota

Precipitated intestinal contents were collected and sent to Biomarker Technologies Co., Ltd. for 16S rRNA gene sequencing analysis using the Illumina PE300/PE250 platform. The structure, function, dynamics, and relationship with the environment or host of microbial communities were comprehensively revealed through OTU analysis, phylum-level classification, genus-level classification, and characteristic microorganism analysis.

2.19. Statistical Analysis

Data analysis was performed using SPSS 26.0 software. Experimental data are expressed as mean $\pm$ SD. *t*-tests were used for comparisons between two groups. Normality and homogeneity of variance were assessed using Levene's test. If assumptions of normality and homogeneity of variance were met, one-way ANOVA was used for statistical analysis; if not, the Kruskal–Wallis test was applied. If the Kruskal–Wallis test was statistically significant ($p < 0.05$), Dunnett's test was used for post hoc comparison analysis.

3. Results

3.1. Characterization of MCE-Lips

Visual inspection showed that MCE-Lips formed a clear, transparent, orange–yellow solution without any precipitation (Figure 1A). Transmission electron microscopy (TEM) images revealed that the MCE-Lips were spherical in shape, with a particle size of approximately 90.7 nm, uniform in size, and free of aggregation (Figure 1B), indicating successful preparation. As measured by laser particle sizing, the average particle size was 86.49 ± 0.67 nm (Figure 1D), with a PDI of 0.128 ± 0.002 (Figure 1E) and a zeta potential of 1.19 ± 0.166 mV (Figure 1F). These results demonstrate a highly uniform size distribution and a stable preparation process. Analysis of MCE-Lips prepared under optimal conditions showed a drug loading of $(1.60 \pm 0.03)\%$ and a high encapsulation efficiency of $(89.07 \pm 2.01)\%$. Stability evaluation (Figure 1G) indicated that with prolonged storage, both the average particle size and PDI generally exhibited an increasing trend. The increase in particle size was accompanied by a decrease in dispersion stability, suggesting possible particle aggregation, which may be attributed to the relatively low zeta potential.

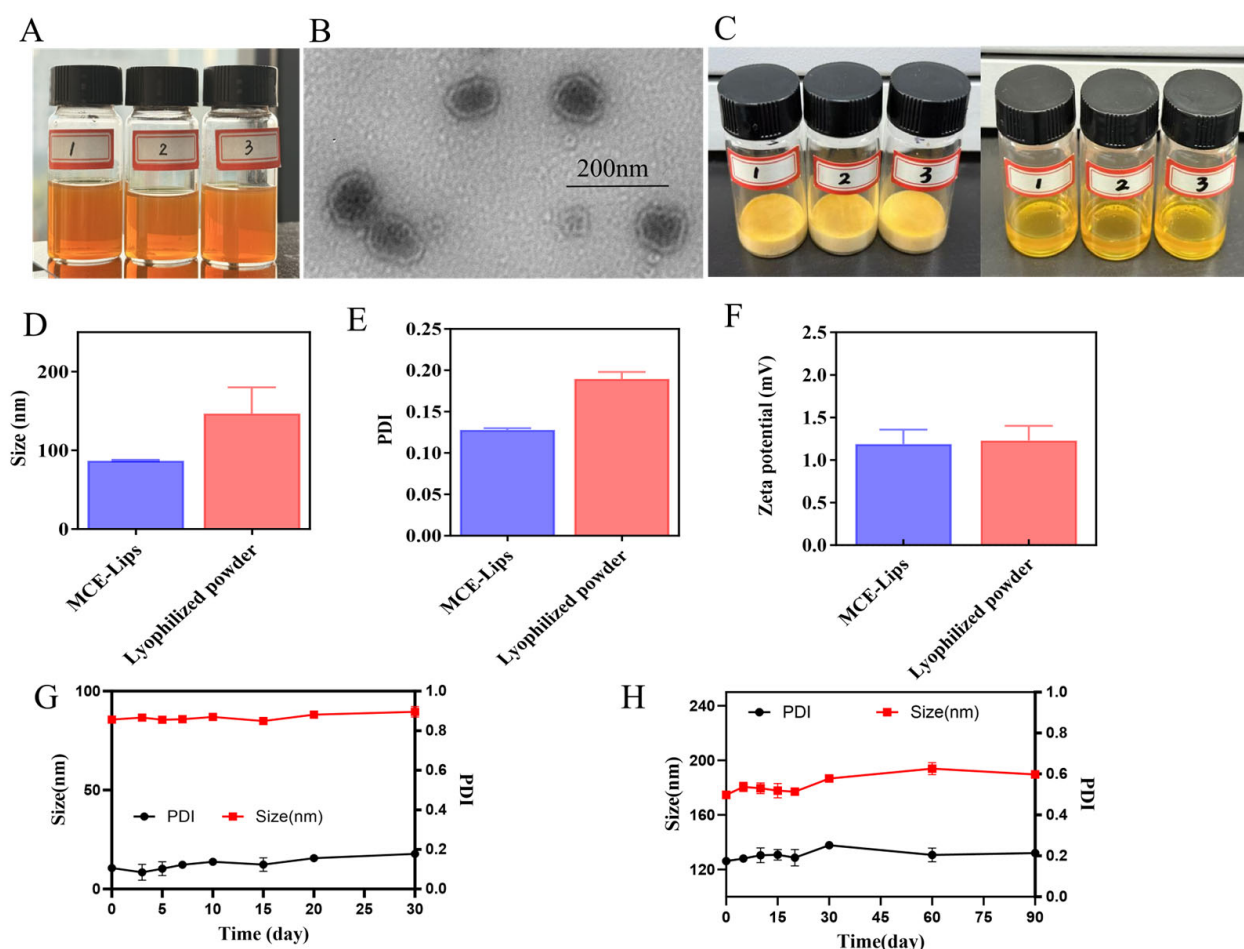


Figure 1. Physicochemical properties of MCE-Lips and lyophilized powder. (A) Appearance of MCE-Lips. (B) TEM image of MCE-Lips (scale 200 nm). (C) Appearance of lyophilized MCE-Lips and its reconstituted form. (D) Average particle size of MCE-Lips and lyophilized powder. (E) Polydispersity index (PDI) of MCE-Lips and lyophilized powder. (F) Zeta potential of MCE-Lips and lyophilized powder. (G) Stability results of MCE-Lips. (H) Stability results of lyophilized MCE-Lips powder.

3.2. Characterization of MCE-Lips Lyophilized Powder

The lyophilized powder prepared through freeze-drying had a smooth and fine appearance. After reconstitution, the solution became clear and transparent again (Figure 1C). The lyophilized formulation exhibited a zeta potential of 1.23 ± 0.171 mV, an average particle size of 177.2 ± 0.51 nm, and a PDI of 0.19 ± 0.008 , indicating a uniform particle size distribution. The drug loading of the lyophilized powder was determined to be 2.82 mg/g. Moreover, the lyophilization process successfully maintained the physical stability of MCE-Lips, making the resulting powder suitable for long-term storage (Figure 1H).

3.3. In Vitro Skeletal Muscle Cell Cytotoxicity

In vitro cytotoxicity assays compared the effects of ordinary injection formulations versus MCE-Lips on cells. After 24 h, the ordinary injection formulation exhibited significant toxicity at a concentration of $1.25 \mu\text{g/mL}$ ($p < 0.0001$), whereas MCE-Lips showed no significant damage ($p > 0.05$) (Figure 2A,B). Therefore, $1.25 \mu\text{g/mL}$ was selected for LDH and inflammatory cytokine assays. These results indicate that the liposomal structure confers superior cellular compatibility to MCE-Lips, effectively reducing cytotoxicity at lower concentrations.

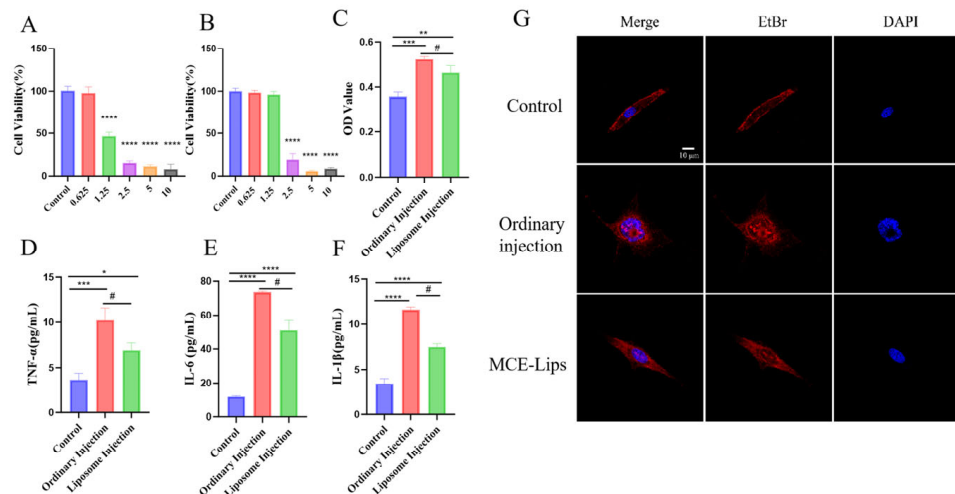


Figure 2. Protective and anti-inflammatory effects of MCE-Lips on skeletal muscle cells. (A) ordinary injection, treatment 24 h. (B) MCE-Lips treatment 24 h. (C) LDH release. (D) TNF- α . (E) IL-6. (F) IL-1 β release levels. (G) Cell membrane rupture results. Compared to control group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Compared to ordinary group, # $p < 0.05$.

3.4. Effects of MCE-Lips on Lactate Dehydrogenase Activity, Inflammatory Cytokine Release, and Cell Membrane Protection in Skeletal Muscle Cells

LDH is a stable cytoplasmic enzyme widely distributed across various organisms, and its release directly reflects the extent of cell membrane damage [30]. Upon cellular injury or death, LDH is released into the extracellular space. Cell membrane rupture leads to the abnormal release of inflammatory mediators, such as IL-1 β and TNF- α , thereby triggering tissue damage and inflammatory cascade reactions [31]. Figure 2C–F demonstrates that compared to the control group, the standard injection solution significantly increased LDH release and elevated levels of inflammatory cytokines IL-1 β , TNF- α , and IL-6, resulting in cellular swelling and increased membrane permeability. In contrast, the MCE-Lips group significantly reduced LDH release ($p < 0.05$) and decreased levels of IL-1 β , TNF- α , and IL-6 ($p < 0.05$), improved membrane permeability, maintained membrane integrity, and preserved the elongated spindle-shaped morphology of cells (Figure 2G). This indicates that compared to ordinary injection, MCE-Lips significantly reduces cellular damage and inflammatory stimulation and better preserves normal cell morphology and membrane integrity, confirming its lower cytotoxicity and superior biocompatibility.

3.5. In Vitro NCM460 Cell Viability Assay and the Effect of LPS Stimulation on Inflammatory Factor Production

As shown in Figure 3A,B, within the concentration range of 0.63–10 $\mu\text{g/mL}$, ordinary injection significantly inhibited NCM460 cell viability ($p < 0.0001$), whereas MCE-Lips showed no significant effect at 0.63 $\mu\text{g/mL}$ ($p > 0.05$), demonstrating superior safety. Under LPS stimulation, both the ordinary injection and MCE-Lips further enhanced cell proliferation ($p < 0.01$ and $p < 0.001$), with MCE-Lips exhibiting a more pronounced effect. This indicates that both formulations improve cell survival in an LPS-induced inflammatory environment. Further anti-inflammatory experiments revealed that compared to the ordinary injection, MCE-Lips exhibited a more pronounced inhibitory effect on LPS-induced TNF- α ($p < 0.01$), IL-6, and IL-1 β ($p < 0.0001$) release, confirming its superior protective and anti-inflammatory efficacy in inflammatory conditions.

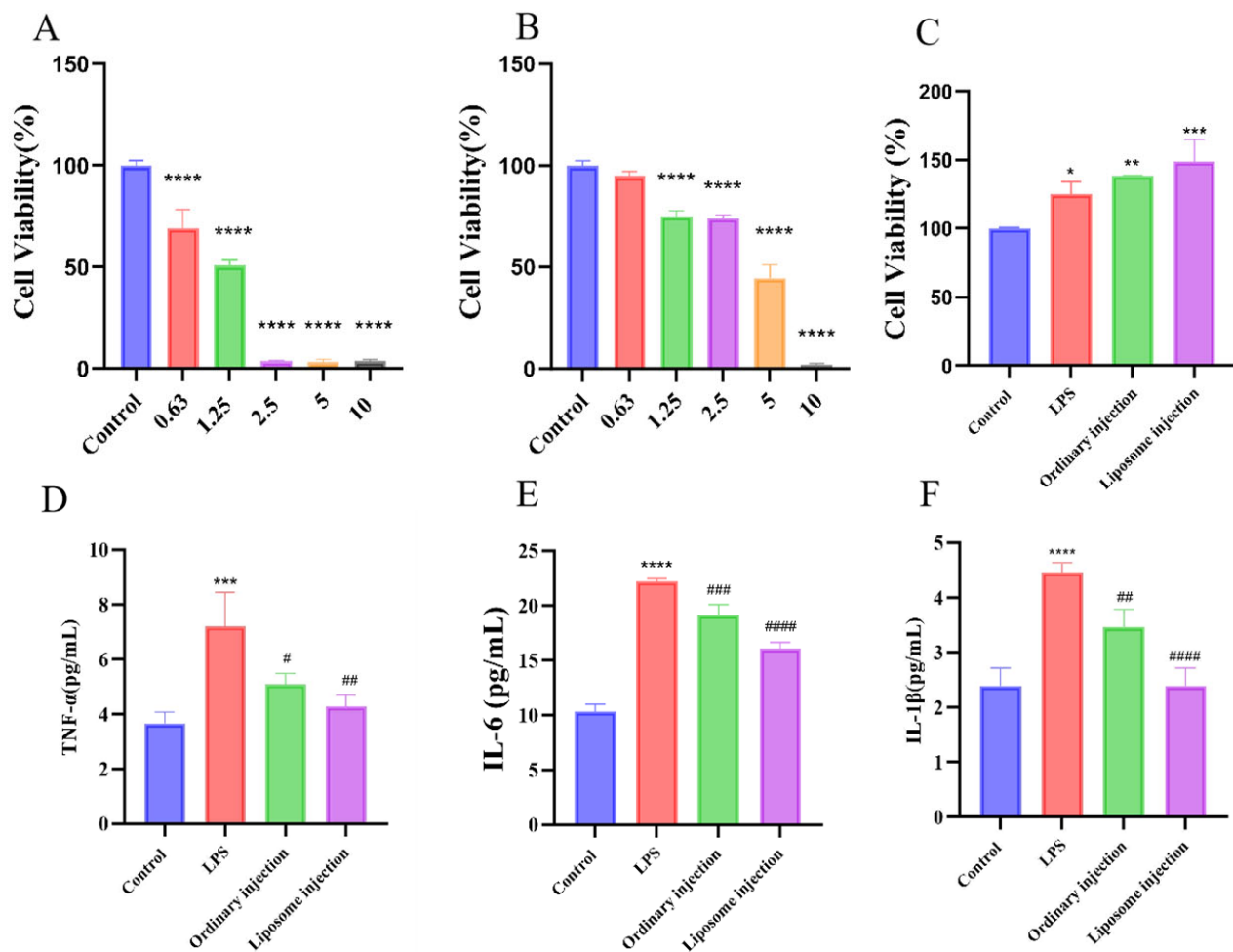


Figure 3. Effects of different drugs on NCM460 cell viability and inhibition of inflammatory factor release. (A) Effect of ordinary injection on NCM460 cell viability after 24 h treatment. (B) Effect of MCE-Lips on NCM460 cell viability after 24 h treatment. (C) Effect of drugs combined with LPS on NCM460 cytotoxicity. (D) TNF- α . (E) IL-6. (F) IL-1 β release levels. Compared to control group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Compared to LPS group, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$.

3.6. Effect of MCE-Lips on Tight Junction Protein (ZO-1, Occludin, Claudin-5) Expression

Immunofluorescence was used to directly evaluate the expression of key intestinal barrier tight junction proteins (ZO-1, Occludin, Claudin-5). As shown in Figure 4, compared to the blank group, the fluorescence intensity of ZO-1, Occludin, and Claudin-5 was significantly weakened in the model group. Both the ordinary injection group and the MCE-Lips group increased the expression of these proteins compared to the model group. However, the fluorescence intensity of ZO-1 ($p < 0.001$), Occludin ($p < 0.01$), and Claudin-5 ($p < 0.01$) in the MCE-Lips group was significantly higher than in the ordinary injection group. This indicates that MCE-Lips enhances intestinal mucosal barrier function by significantly upregulating the expression of ZO-1, Occludin, and Claudin-5, exerting intestinal protection and anti-inflammatory effects. This finding is consistent with Yue et al. [32], suggesting restoration of intestinal barrier integrity by promoting tight junction protein expression.

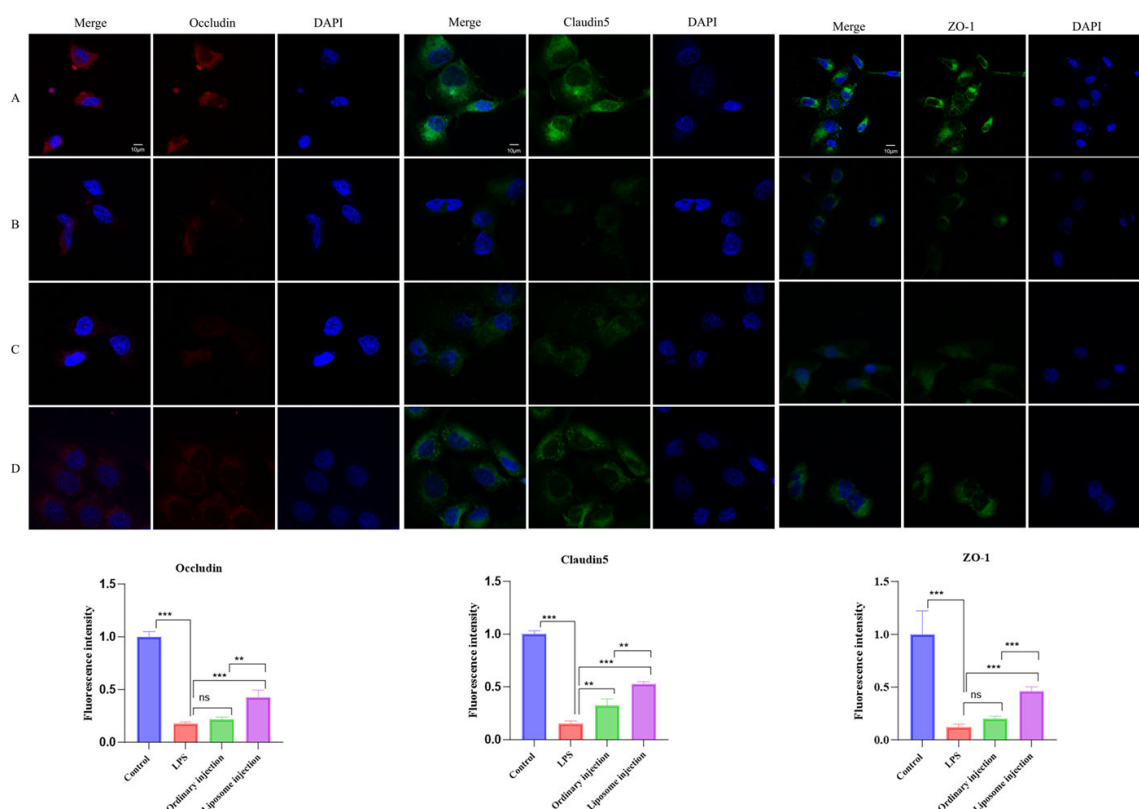


Figure 4. Effects of ordinary injection and MCE-Lips on the expression of ZO-1, Occludin, and Claudin-5 proteins. (A) Blank control group. (B) Model group. (C) Ordinary injection group. (D) MCE-Lips group. Compared with the control group, * $p < 0.01$, ** $p < 0.001$, ns, no significant difference.

3.7. Acute Toxicity and Anti-Diarrheal Efficacy

Given the known toxicity of MCE, evaluating the safety of MCE-Lips is crucial. Within 0–8 h after administration, the general state (spontaneous activity, eating, defecation) of mice in the MCE-Lips group and the blank group was normal, with no other toxic reactions observed. During continuous observation for 14 days, all mice showed no abnormalities or deaths. As shown in Figure S1, the weight gain trend of mice in the MCE-Lips group was consistent with that of the blank group. At the maximum administered dose, MCE-Lips did not significantly inhibit mouse weight gain, with no statistically significant difference between the two groups ($p > 0.05$). H&E results indicate that MCE-Lips did not exhibit acute toxic reactions in the mouse model (Figure S2).

In the bacterial diarrhea model, the safety-validated MCE-Lips demonstrated clear efficacy (Figure 5 and Table 2). Compared to the blank group, the diarrhea latent time in the model group was significantly shortened ($p < 0.01$), and the diarrhea index in both the 0–2 h and 2–4 h periods was significantly increased ($p < 0.01$). Compared to the model group, the medium and high-dose MCE-Lips groups significantly prolonged the diarrhea latent time ($p < 0.05$) and significantly reduced the diarrhea index in both the 0–2 h and 2–4 h periods ($p < 0.01$), showing a dose-dependent effect. The Chuanxinlian injection group significantly reduced the diarrhea index in both the 0–2 h and 2–4 h periods ($p < 0.01$). In the 2–4 h period, all MCE-Lips dose groups significantly reduced the diarrhea index ($p < 0.01$), suggesting that efficacy stabilizes with prolonged intervention time. These results indicate that medium and high doses of MCE-Lips exert a clear anti-diarrheal effect by significantly prolonging diarrhea latent time and reducing the diarrhea index in a dose-dependent manner.

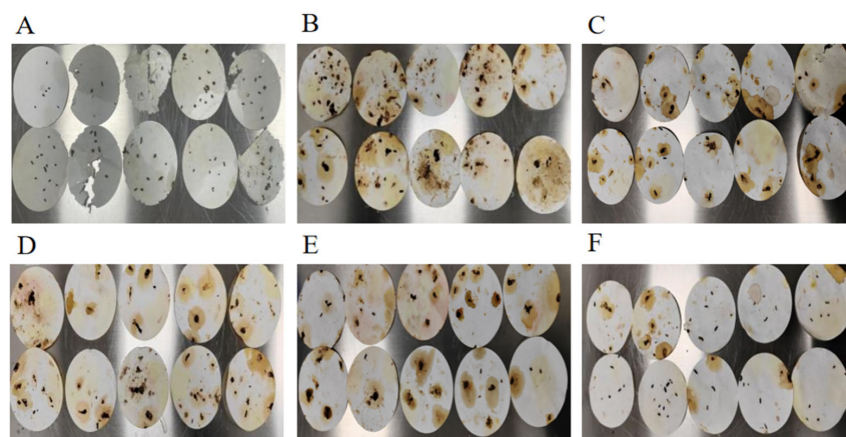


Figure 5. Mouse defecation status. (A) Blank group. (B) Model group. (C) Chuanxinlian injection group. (D) MCE-Lips low-dose group. (E) MCE-Lips medium-dose group. (F) MCE-Lips high-dose group.

Table 2. Effect of MCE-Lips on bacterial diarrhea in mice.

Group	Incidence of Diarrhea	Diarrhea Incubation Time (min)	Diarrhea Index	
			0–2 h	2–4 h
Blank group	0/10	240.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Model group	10/10	32.80 ± 10.38 **	2.50 ± 1.18 **	3.60 ± 0.52 **
Chuanxinlian injection	9/10	76.00 ± 63.80 **,#	0.70 ± 0.48 **,##	1.40 ± 0.84 **,##
Low MCE-Lips	10/10	34.80 ± 19.18 **	1.40 ± 1.17 **	2.30 ± 0.82 **,##
Medium MCE-Lips	9/10	68.20 ± 63.47 **,#	0.50 ± 0.71 ##	1.50 ± 0.97 **,##
High MCE-Lips	8/10	92.90 ± 82.00 **,#	0.60 ± 0.70 ##	1.30 ± 1.16 **,##

Note: Compared with the blank group, ** $p < 0.01$. Compared with the model group, # $p < 0.05$, ## $p < 0.01$. The data are presented as mean ± SD ($n = 10$).

3.8. Effect of MCE-Lips on Mouse Hematology

To investigate if the anti-diarrheal effect of MCE-Lips involved immunomodulation, hematological tests showed no significant differences between treatment groups and the model group in red blood cell count, hemoglobin concentration, platelet count, or granulocyte subpopulation proportions (neutrophils, lymphocytes, monocytes) ($p > 0.05$) (Table 3). This indicates that MCE-Lips did not induce systemic toxic reactions within the experimental dose range, demonstrating good biosafety. The medium-dose group significantly inhibited model-induced leukocytosis ($p < 0.05$), suggesting it may alleviate tissue damage by regulating inflammatory responses.

Table 3. Effects of MCE-Lips on various indicators of mouse blood.

Group	Blank Group	Model Group	Chuanxinlian Injection	Low MCE-Lips	Medium MCE-Lips	High MCE-Lips
WBC ($10^9/L$)	0.83 ± 0.21	2.46 ± 1.46 **	1.58 ± 1.04	2.16 ± 1.48	1.54 ± 0.43 #	1.47 ± 0.60
NEUT (%)	20.48 ± 8.82	19.37 ± 6.27	23.44 ± 11.00	20.50 ± 8.75	19.87 ± 6.15	24.80 ± 6.66
Lymph (%)	73.40 ± 11.89	76.87 ± 8.18	69.70 ± 13.64	75.81 ± 11.90	76.86 ± 8.18	69.77 ± 8.29
Mono (%)	4.69 ± 3.10	3.17 ± 2.60	5.18 ± 3.34	3.07 ± 3.08	2.61 ± 2.54	4.56 ± 4.09
Eos (%)	1.00 ± 0.67	0.40 ± 0.35 *	1.22 ± 1.01 #	0.32 ± 0.19	0.25 ± 0.18	0.58 ± 0.52
Baso (%)	0.43 ± 0.38	0.19 ± 0.16	0.46 ± 0.53	0.30 ± 0.25	0.41 ± 0.29	0.29 ± 0.19

Table 3. Cont.

Group	Blank Group	Model Group	Chuanxinlian Injection	Low MCE-Lips	Medium MCE-Lips	High MCE-Lips
RBC (10 ¹² /L)	6.02 ± 0.42	5.88 ± 0.35	5.92 ± 0.84	6.19 ± 0.40	5.89 ± 0.41	6.07 ± 0.51
HGB (g/L)	117.00 ± 8.15	115.90 ± 4.91	115.60 ± 16.10	118.60 ± 7.62	113.90 ± 7.46	118.70 ± 7.47
PLT (10 ⁹ /L)	814.90 ± 96.98	866.20 ± 141.76	752.40 ± 133.27	866.90 ± 128.95	753.00 ± 177.72	774.80 ± 208.95

Note: Compared with the blank group, * $p < 0.05$, ** $p < 0.01$. Compared with the model group, # $p < 0.05$. The data are presented as mean ± SD ($n = 10$).

3.9. Effect of MCE-Lips on Inflammatory Factors IL-6 and TNF- α and Gastrointestinal Hormones

Combined with the hematology results, the detection of serum inflammatory factors and gastrointestinal hormones further revealed the mechanism. When inflammation occurs, immune cells release cytokines IL-6 and TNF- α , whose levels reflect the degree of inflammation. Table 4 shows that low, medium, and high doses of MCE-Lips had no significant effect on IL-6 or TNF- α ($p > 0.05$). Medium and high doses downregulated the acute-phase protein CRP ($p < 0.05$), suggesting that its anti-diarrheal mechanism is related to inhibiting acute inflammatory responses rather than directly intervening in pro-inflammatory cytokine pathways. At the endocrine level, the high dose significantly reduced motilin (MTL) ($p < 0.01$), and low and medium doses also showed inhibitory trends ($p < 0.05$). Decreased MTL can slow intestinal peristalsis and prolong chyme retention, thereby alleviating diarrhea.

Table 4. Effect of MCE-Lips on inflammatory factors and hormones.

Group	IL-6	TNF- α	CRP	MTL	GAS
Blank group	398.64 ± 106.67	2201.01 ± 229.56	27.22 ± 2.10	166.77 ± 17.96	363.95 ± 20.47
Model group	533.00 ± 47.41 **	2617.38 ± 186.93 **	33.53 ± 3.63 **	339.03 ± 68.46 **	285.79 ± 70.44
Chuanxinlian injection	436.01 ± 52.48 ##	2334.59 ± 225.03 ##	30.18 ± 1.12 #	261.56 ± 35.93 ##	350.70 ± 37.63
Low MCE-Lips	491.02 ± 82.65	2548.25 ± 153.78	31.17 ± 1.76 #	295.52 ± 65.98 #	292.96 ± 100.79
Medium MCE-Lips	468.54 ± 76.83	2473.25 ± 274.53	31.03 ± 1.36 #	283.07 ± 38.38 #	316.23 ± 54.83
High MCE-Lips	464.08 ± 83.87	2426.00 ± 208.12	30.60 ± 1.99 #	270.50 ± 15.36 ##	344.65 ± 29.76

Note: Compared with the blank group, ** $p < 0.01$. Compared with the model group, # $p < 0.05$, ## $p < 0.01$. The data are presented as mean ± SD ($n = 10$).

3.10. MCE-Lips Ameliorated Fecal Metabolite Dysbiosis in Diarrheal Mice

UPLC-QTOF/MS analysis indicated that MCE-Lips improved fecal metabolite dysregulation. Non-targeted metabolomics analysis identified the following results. Table S1 summarizes the fecal metabolites detected in the KB (blank) and WN (MCE-Lips treated) groups. A total of 2311 substances were detected between the two groups, including 819 in negative ion mode and 1492 in positive ion mode. PCA analysis showed significant separation of the metabolic profiles among the blank group (KB), the model group (CON), and the MCE-Lips treatment group (WN) (Figure 6A). KB and CON groups clustered distinctly into two categories, indicating that MCE-Lips significantly reshaped the host metabolic state. To compare the upregulation and downregulation trends of differential metabolites between groups, key differential metabolites were screened among the three groups based on strict criteria ($p < 0.05$, $VIP \geq 1.5$, $FC \geq 2$) (Table S2) to create volcano plots. Comparing WN vs. CON, 41 differential metabolites were upregulated, and 56 were downregulated in negative ion mode (Figure 6B). Comparing WN vs. KB, 71 metabolites were upregulated and 99 downregulated (107 in negative ion mode, 56 in positive) (Figure 6C). Comparing KB vs. CON, 94 metabolites were upregulated and 86 downregulated (59 in negative ion mode,

120 in positive) (Figure 6D). Among these, organic acids and their derivatives accounted for a high proportion in comparisons of WN vs. KB (45 types), KB vs. CON (30 types), and WN vs. CON (13 types), suggesting that they are core regulatory targets of MCE-Lips.

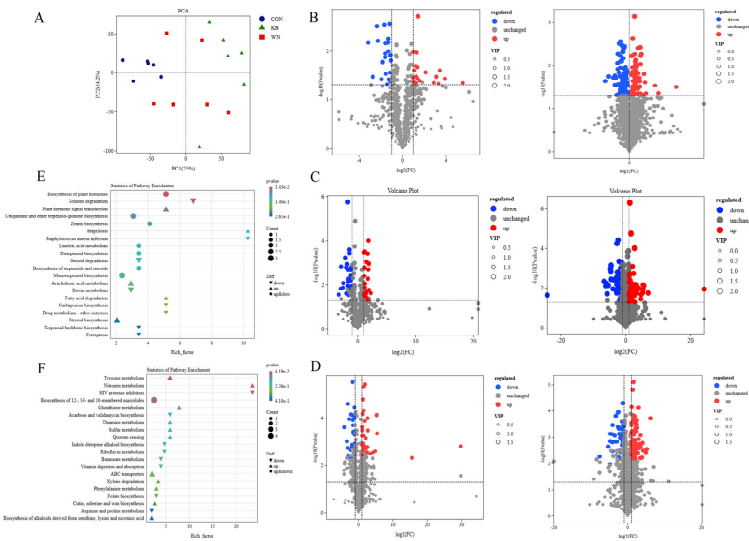


Table 5. Cont.

Group	Differentially Metabolized Substances	p Value	VIP	Log2FC
WN and CON	4-Hydroxyvalproic acid	0.001	2.194	1.93871126412999
	9,12,13-TriHOME	0.024	1.711	−0.797148058
	Choline sulfate	0.033	1.689	3.59177675137981
	5-Hydroxyvalproic acid	0.043	1.595	0.724182683077591

3.11. MCE-Lips Regulated Gut Microbiota Dysbiosis in Diarrheal Mice

After quality control processing, a total of 6789 OTUs were obtained across the three groups (Figure 7A), with 2031 OTUs in the KB group, 2856 in the WN group, and 2695 in the CON group. OTUs differed significantly between the KB and CON groups and between the WN and CON groups, indicating that MCE-Lips significantly altered the intestinal microbiota structure of diarrheal mice. The KB group had the most unique OTUs (1718), suggesting its microbiota uniqueness. Taxonomic composition at the phylum and genus levels was analyzed for each group. Figure 7C shows the dominant phylum composition. *Firmicutes* and *Bacteroidota* were the most abundant phyla in all three groups, with abundance ranking KB > CON > WN, but there was no significant difference between groups ($p > 0.05$). *Proteobacteria* and *Campylobacterota* had very low relative abundance in the KB group, indicating that they were primarily present only in the WN and CON groups. The relative abundance of *Proteobacteria* was significantly higher in WN than in CON (0.193% vs. 0.002%), while *Campylobacterota* abundance was significantly lower in WN than in CON (0.055% vs. 0.094%). Figure 7D focuses on dominant species at the genus level: *Muribaculaceae*, *Lactobacillus*, *Ligilactobacillus*, *Lachnospira*, *Alistipes*, and *Helicobacter*. At the genus level, beneficial bacteria (e.g., *Lactobacillus*) showed significantly increased relative abundance in the WN group (WN > CON > KB), while harmful bacteria (*Alistipes*, *Helicobacter*) abundance decreased. To identify key systemic types and biomarkers of gut microbiota in different groups, LEfSe was used for comparison and analysis of high-dimensional categories (Figure 7B), revealing significant differences in dominant bacterial communities between groups. A total of 15 characteristic microorganisms were screened through LEfSe analysis (LDA score > 3), including seven genera, four families, and four orders.

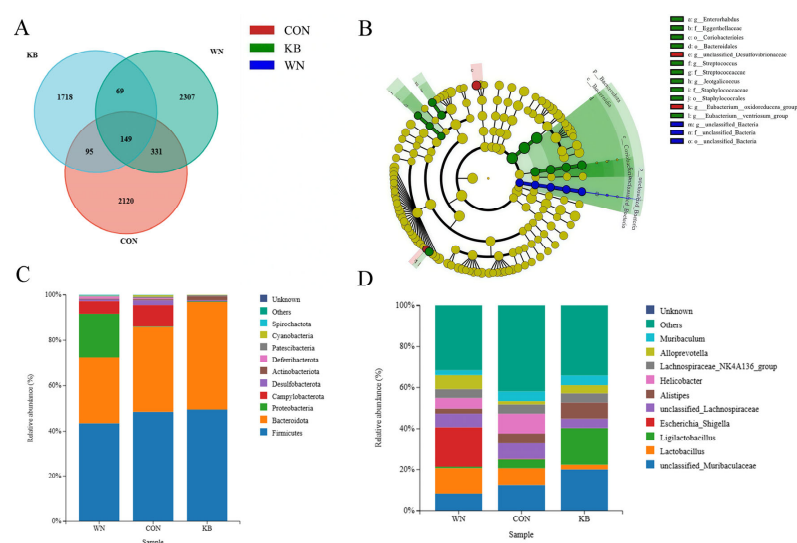


Figure 7. Analysis of microbial community diversity and composition of different groups detected through 16S rRNA sequencing. (A) OTU analysis. (B) Characteristic microorganisms. (C) Phylum-level classification. (D) Genus-level classification (note: WN = MCE-Lips-treated group, KB = blank control, CON = model control).

The results suggest that *Desulfovibrio*, *Enterococcus*, and *Streptococcus* may be characteristic microbiota affected by MCE-Lips.

4. Discussion

Newborn piglets are susceptible to yellow and white scours caused by pathogenic *Escherichia coli* [33], leading to indigestion, stunted growth, and even death. Antibiotics are the primary treatment, but their misuse poses serious threats to the environment and public health. Given MCE's significant antibacterial and anti-inflammatory activity, widely used in the livestock and poultry industry, developing novel natural plant feed additives based on MCE is crucial.

The phospholipid bilayer structure of liposomes enables the simultaneous encapsulation of both hydrophilic and hydrophobic active components from MCE. It may also enhance oral bioavailability by improving intestinal permeability, bypassing first-pass metabolism, and enabling targeted delivery, thereby offering an innovative strategy to overcome the limitations of oral MCE administration [34–36]. In this study, MCE-Lips were prepared using the thin-film dispersion method, resulting in a drug carrier with uniform particle size, favorable stability, and excellent biocompatibility, thereby laying a foundation for in vivo applications. Stability evaluations revealed that the liquid formulation tended to undergo particle size increase and dispersion deterioration during storage, indicating certain limitations to its physical stability. Freeze-drying technology currently serves as an effective approach to extend stability, improve the robustness of liposomal formulations, and facilitate transportation and storage [37,38]. The lyophilized powder obtained through freeze-drying maintained a consistent particle size distribution and physicochemical properties upon reconstitution, significantly enhancing the long-term stability of the formulation and providing convenient conditions for subsequent cellular and animal experiments.

To evaluate the safety of MCE-Lips on skeletal muscle cells and their effects on the intestinal mucosal barrier of colonic epithelial cells, as well as to elucidate the mechanism through which this formulation enhances efficacy and reduces toxicity, we conducted in vitro cell experiments. Both MCE-Lips and the conventional injection formulation caused an increase in LDH release, indicating disruption of cell membrane integrity. Consistent with this, Sun et al. reported that polystyrene nanoparticles also exhibited membrane-disruptive effects, leading to increased LDH release [39]. Comparatively, MCE-Lips induced a lower level of LDH release than the conventional formulation. In line with our findings, Layas et al. demonstrated that resveratrol delivered in liposomal and PLA nanoparticle forms resulted in less cellular damage than the free drug in LDH assays [40]. Previously, our group formulated MCE into an injection and observed significant local adverse reactions in animal muscle tissue (swelling, poor absorption, even darkening and necrosis) during toxicological studies. This study demonstrates that the nano-formulation effectively overcomes the technical bottleneck of local tissue adverse reactions associated with traditional injectable formulations. The cell membrane rupture experiment further confirmed the protective potential of MCE-Lips for skeletal muscle cells.

LPS, a major component of Gram-negative bacterial cell walls, can trigger inflammation in colon epithelial cells like NCM460 by activating pattern recognition receptors (e.g., TLR4) characterized by massive release of pro-inflammatory factors [41]. In infectious colitis, LPS-induced pro-inflammatory factors are central to pathological progression. This study successfully simulated this process and confirmed that MCE-Lips significantly inhibits the release of inflammatory factors in LPS-stimulated NCM460 cells. This is due to the characteristics of its nano carrier, which effectively protects drug activity and significantly increases drug concentration in the local lesion, thus synergistically amplifying the anti-inflammatory effect [42]. Intestinal barrier function is closely related to tight junc-

tion proteins (ZO-1, Occludin, Claudin-5) [43]. In vitro models demonstrate significantly reduced protein expression and compromised barrier function, consistent with the pathological features of inflammatory bowel disease (IBD) [44]. The integrity of intestinal barrier function depends on the structural stability of tight junctions (TJs) between epithelial cells [45]. In this study, treatment with MCE-Lips up-regulated the expression of tight junction proteins ZO-1, Occludin, and Claudin-5, indicating their ability to effectively repair tight junction (TJ) structure, enhance intestinal barrier function, and reduce the permeation of inflammatory mediators. This finding reveals their potential value in the treatment of colitis.

In an acute toxicity study, Dong et al. reported that the LD50 of MCE in mice was 1024.33 mg/kg, with no significant reproductive or embryonic toxicity observed [46]. In the present study, administration of MCE-Lips at a maximum dose of 76.85 mg/kg did not induce abnormal changes in body weight or cause pathological organ damage in mice, indicating a superior safety profile compared to free MCE. These findings suggest that the MCE-Lips carrier may enhance safety by improving drug distribution characteristics or reducing potential irritation associated with the rapid release of the free drug.

To deeply explore the mechanism of efficacy enhancement in diarrhea treatment, an in vivo bacterial diarrhea model was employed. For the first time, non-targeted metabolomics and 16S rRNA technology were combined to investigate the mechanism of MCE-Lips against diarrhea caused by bacterial infection. Non-targeted metabolomics, using techniques like UPLC-QTOF-MS, comprehensively analyzed the intestinal metabolic profile of diarrheal mice and identified core differential metabolites: 4-Hydroxyvalproic acid, 9,12,13-TriHOME, Choline sulfate, and 5-Hydroxyvalproic acid. Among them, valproic acid derivatives can regulate secretion and inflammation [47] and are also classic histone deacetylase inhibitors [48]. By inhibiting HDAC activity, they activate anti-inflammatory gene transcription, block pro-inflammatory signals like NF- κ B, reduce the release of intestinal pro-inflammatory factors like IL-6 and TNF- α , and ameliorate infectious diarrhea [49]. TriHOME maintains osmotic pressure and microbiota homeostasis. Choline sulfate promotes repair and neuromodulation [50] and acts as a mediator in the cholinergic anti-inflammatory pathway, inhibiting vagus nerve-macrophage inflammasome activation [51]. This indicates that MCE-Lips may exert their therapeutic effect on mouse diarrhea primarily by influencing the metabolic levels of these four substances.

The 16S rRNA sequencing results further demonstrate that MCE-Lips act as a “regulator” of the gut microbiota in diarrheal mice. They remodel the microbiota structure, specifically promoting beneficial bacteria like *Lactobacillus*, which plays a vital role in maintaining the health of humans and animals. Nutritionally, *Lactobacillus* can improve protein digestion [52] and inhibit harmful bacteria like *Helicobacter*, which can cause chronic gastritis, peptic ulcers, and other diseases [53], thereby creating a healthier and more stable intestinal microecological environment, directly linked to its efficacy in treating diarrhea.

This study, for the first time, developed MCE-Lips to achieve the dual goal of “enhancing efficacy while reducing toxicity.” The anti-diarrheal effects and mechanisms of MCE-Lips were systematically evaluated through multi-dimensional experiments (cellular, animal, metabolomic, and microbiome analyses). By integrating untargeted metabolomics and 16S rRNA sequencing, the potential mechanisms of MCE-Lips involving multi-pathway synergistic actions were revealed. The formulation process is simple and reproducible, and the lyophilized powder form facilitates storage and transportation, demonstrating promising translational potential. However, this study is limited by the use of a mouse model, whose intestinal physiology and immune system differ from those of piglets, necessitating further validation in porcine models. The metabolomic and microbiome analyses are correlative in nature; although they suggest potential mechanisms, causal relationships have

not been verified through gene knockout or inhibitor experiments. Although liposomes alleviated myotoxicity, their targeting efficiency in inflamed intestines and intracellular delivery mechanisms still require further investigation.

In summary, this study successfully developed MCE-Lips, which possess favorable physicochemical properties and good biocompatibility. MCE-Lips exert their therapeutic effects through multiple pathways, including enhancing the intestinal barrier, modulating microbial composition, suppressing inflammatory responses, and regulating gastrointestinal motility. Future work should focus on validating the efficacy, elucidating the underlying mechanisms, and evaluating the long-term safety of MCE-Lips in piglet models to advance their translational development toward veterinary drug applications.

5. Conclusions

In summary, our study successfully developed and characterized MCE-loaded nanoliposomes (MCE-Lips) with uniform particle size, favorable stability, and excellent biocompatibility. By innovatively integrating nanotechnology with natural bioactive compounds, we provide an efficient and safe delivery strategy to overcome the clinical application barriers of MCE. This work establishes a theoretical foundation and technical pathway for developing natural compound-based nano-formulations to prevent and treat bacterial diarrhea in piglets. Future studies should focus on investigating the release kinetics of SA and CHE from MCE-Lips via dialysis experiments, as well as evaluating their long-term efficacy in livestock models.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/pharmaceutics18020218/s1>, Figure S1: Mouse body weight change; Figure S2: Histopathological sections of major organs (H&E staining, 40×, $n = 10$); Figure S3: HPLC chromatogram map; Figure S4: Experimental Procedure Flowchart. Table S1: Metabolite classification; Table S2: Differentiated metabolite categories in each group.

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Institutional Review Board Statement: The animal experiments in this study were conducted in compliance with the regulations of the Animal Experiment Ethics Committee of the Hunan University of Traditional Chinese Medicine, located in Hunan, China (HNUCM11-2503-050, approval date: 11 February 2025).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MCE	<i>Macleaya cordata</i> extract
MCE-Lips	<i>Macleaya cordata</i> extract-Liposome
SA	Sanguinarine
CHE	Chelerythrine
DHSA	Dihydrosanguinarine
DHCHE	Dihydrochelerythrine
IM	Intramuscular
UPLC-MS/MS	Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry
TEM	Transmission electron microscope
LDH	Lactic dehydrogenase
UPLC-QTOF-MS	Ultra Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry
Etbr	Ethidium bromide
TJs	Tight Junctions
TNF- α	Tumor necrosis factor alpha
IL-1 β	Interleukin-1 β
IL-6	Interleukin-6
16S rRNA	16S ribosomal ribonucleic acid
PCA	Principal component analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes

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