Supplementary Materials

Phlorotannin Nanoparticle-Hydrogel Composite for Enhanced Oral Delivery and Treatment of Ulcerative Colitis

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2. Materials and methods

2.1 Characterizations of nanoparticles

PT NPs morphologies were observed using a JSM-7800F scanning electron microscope (SEM) and a JEM2100F transmission electron microscope (TEM) (JEOL, Japan). The elemental content of PT NPs was quantified by X-Max 50 energy dispersive spectroscopy (EDS) (Oxford Instruments, UK). The structures of PT and PT NPs were analyzed by infrared spectroscopy (PerkinElmer Spectrum Two FT-IR spectrometer, Japan). A UV-visible spectrophotometer (LAMBDA 35, Japan) with a wavelength range of 190 to 700 nm was used to measure the UV-visible spectra of PT and PT NPs. The crystal structures of PT and PT NPs were analyzed by XRD-7000 X-ray diffractometer (XRD) (Shimadzu, Japan). Particle size, zeta potential, and PDI of PT NPs were measured using a ZS XPLORER laser particle size analyzer. Additionally, PT concentrations ranging from 0.5 to 2.5 mg/mL were used to prepare different concentrations of PT NPs. UV and XRD analyses were performed to investigate whether different concentrations of PT would affect the properties of the formed nanoparticles.

2.2 Stability of nanoparticles

The stability of PT NPs was evaluated under various conditions. For storage stability, PT NPs dispersion was prepared, and the particle size and polydispersity index (PDI) were measured at 0, 2, 4, 6, and 8-day intervals. For salt ion stability, PT NPs were dissolved in 0, 0.1, 0.2, 0.3, and 0.4 M NaCl solutions. After 1 h at room temperature, the particle size and PDI were measured using a ZS XPLORER laser particle size analyzer (Malvern Instruments Ltd.). Photothermal stability was assessed through photostability and thermal stability tests. For photostability, PT NPs solution bottles were placed in a UV radiation chamber, and PT content was determined at various time

intervals. For thermal stability, PT NPs solutions were placed in a water bath at different temperatures (25, 40, 50, 60, 70, and 80 °C) for 12 h. PT content was determined using a microplate reader after cooling to room temperature.

2.3 Rheological Characterization

A TA Rheometer was used to test the rheological properties of the hydrogels. A 20 mm parallel plate fixture was chosen for the tests and the gap of the measurement cell was set at 1.0 mm. The rheometer temperature was set at 25 °C to measure the change in viscosity with shear rate. Shear rates ranged from approximately 1 s-1 to 100 s-1. Rheological profiles were analysed by plotting viscosity-shear rate curves.

2.4 Cell Culture

RAW264.7 cells were incubated at 37°C in a 5% CO_2 incubator. DMEM medium supplemented with antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin) and serum (v/v=1:10) was used to culture the cells.

2.5 Evaluation of cellular uptake capacity

RAW264.7 cells were inoculated into containers (1×10^6 cells/well) and cultured for 24 h. The cells were then treated with FITC-labelled PT, PT NPs, and PT NPs-Gel for 3 h. followed by PBS washing. They were then fixed with 4% paraformaldehyde for 15 min and washed twice with PBS. Subsequently, DAPI was used to stain the cell nuclei for 15 min and washed again with PBS. Qualitative and quantitative analysis of cell uptake using confocal laser microscopy (SP8, Leica, Wetzlar, Germany) and flow cytometry (FACSVerse, BD, USA).

2.6 Animals

Male Balb/c mice (6-8 weeks of age) were purchased from Liaoning Changsheng Biotechnology

Co., Ltd. (Benxi, China). All animal managements and experimental protocols were approved by the Experimental Animal Ethics Committee of the National Engineering Research Center of Seafood of Dalian Polytechnic University (Dalian, China; Animal Ethics Review Approval Number: DLPU2023071). Before the experiment, the mice were acclimatized and fed for 7 days.

2.7 Biological distribution

First, FITC-loaded PT NPs (FITC NPs) and PT NPs-Gel (FITC NPs-Gel) were prepared by the method for the preparation of PT NPs, with FITC instead of PT. Balb/c mice were randomly divided into three groups. After acclimatization for 7 days of feeding, a UC model was established. The mice were given distilled water containing 3% DSS for 7 consecutive days. FITC, FITC NPs, and FITC NPs-Gel were orally administered to the respective groups. The mice were then euthanized and their intestines were taken at specific time points (2, 6, 12, 24 h). A multifunctional *in vivo* imager (MIIS XFP-BIX, Molecular Devices, USA) was used to evaluate the biodistribution of the different substances in the intestine.

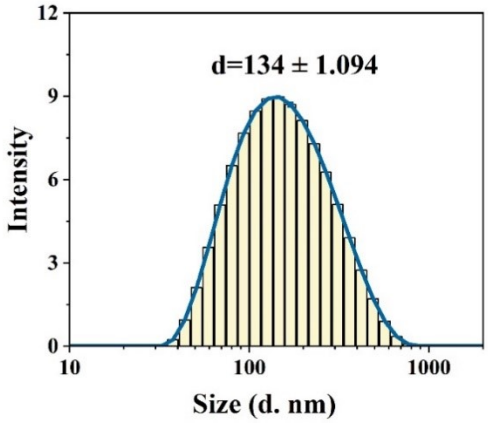


Fig. S1 Particle size distribution of PT NPs.

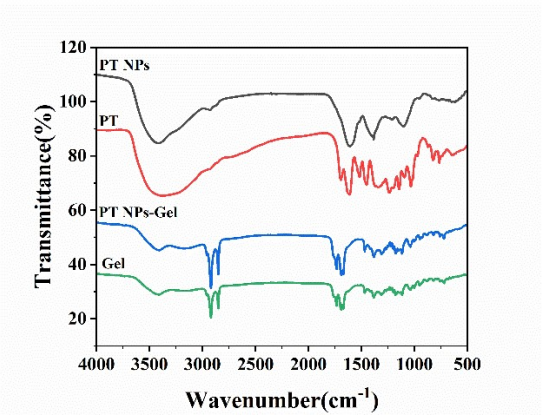


Fig. S2. Infrared spectra of different groups of samples: PT, PT NPs, PTNPs-Gel and Gel.

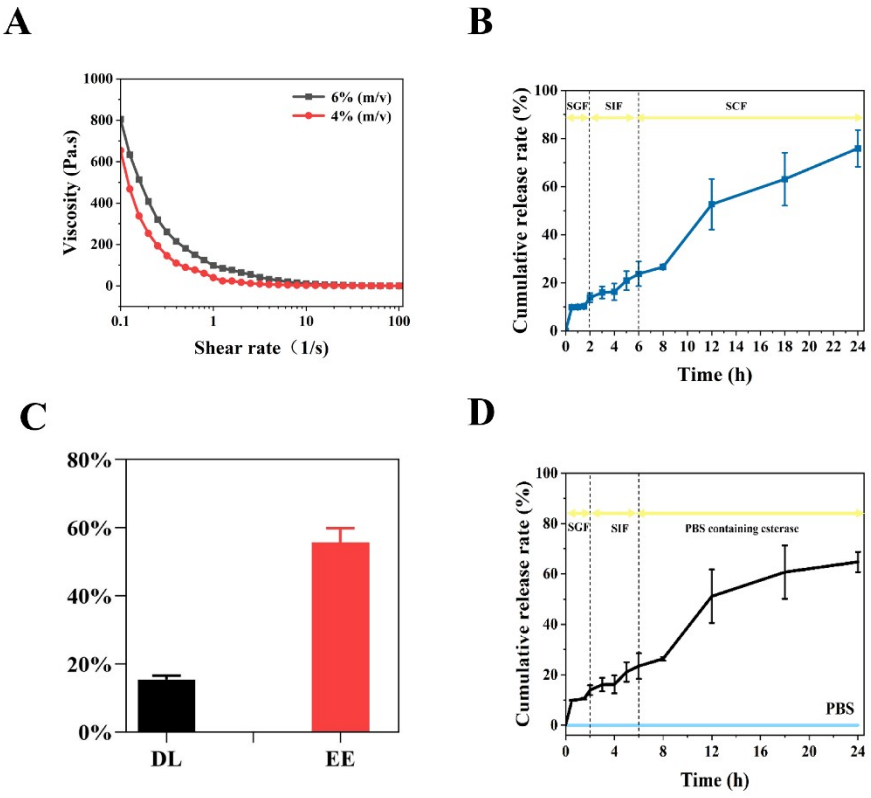
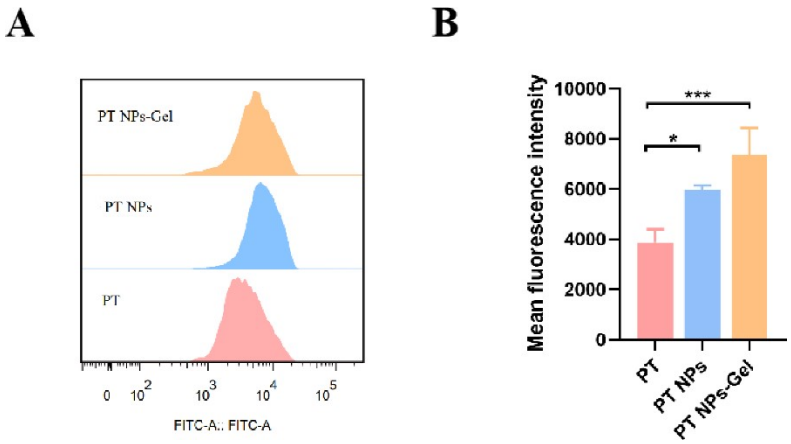


Fig. S3 Characterization of PTNPs-Gel. (A) Rheological properties of hydrogels with different concentrations. (B) Release of PT from PTNPs-Gel *in vitro*. (C) Loading efficiency and encapsulation efficiency of PTNPs-Gel. (D) Cumulative release of hydrogels in PBS vs. PBS containing esterase. Esterase (≥20units/mg solid, porcine liver) was added at hour 6.



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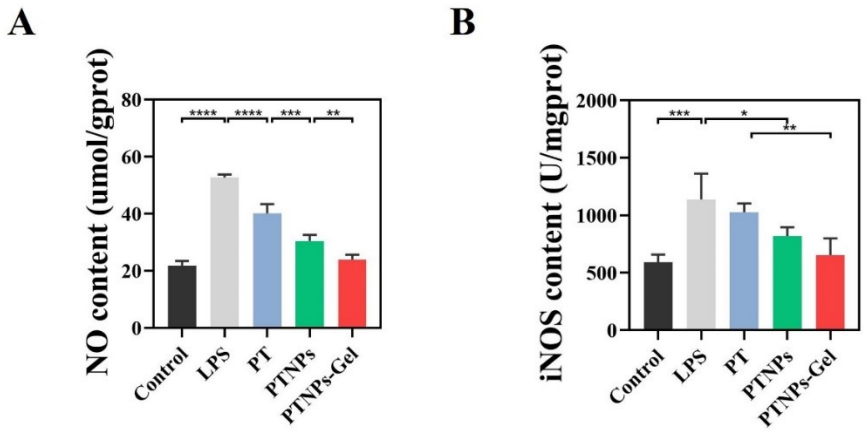
82 Fig. S4 RAW264.7 Quantitative cellular uptake of PT, PTNPs, PTNPs-Gel by RAW264.7cells.
83 (n=3)



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85 Fig. S5 The levels of (A) NO and (B) iNOS in RAW264.7 cells treated with different samples.
86 The data are expressed as means ± SD (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p <$
87 0.0001.

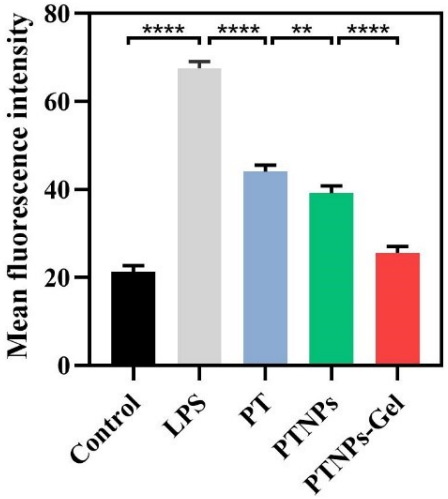

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Fig. S6 Average fluorescence intensity of intracellular ROS in RAW264.7 cells treated with PT, PT NPs, and PT NPs-Gel. The data are expressed as means ± SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

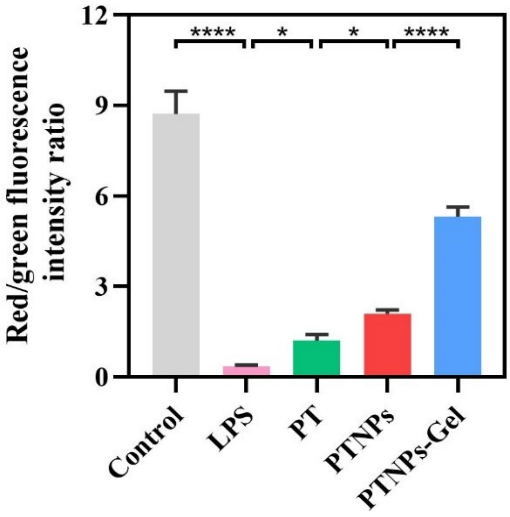


Fig. S7 The ratio of red/green fluorescence intensity in RAW264.7 cells treated with PT, PT NPs, and PT NPs-Gel. The data are expressed as means ± SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

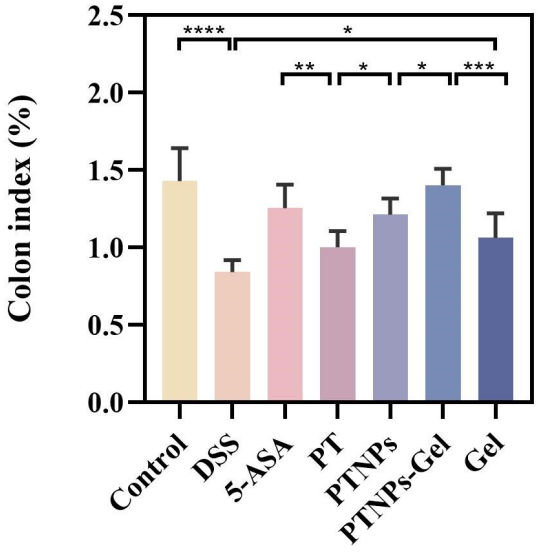
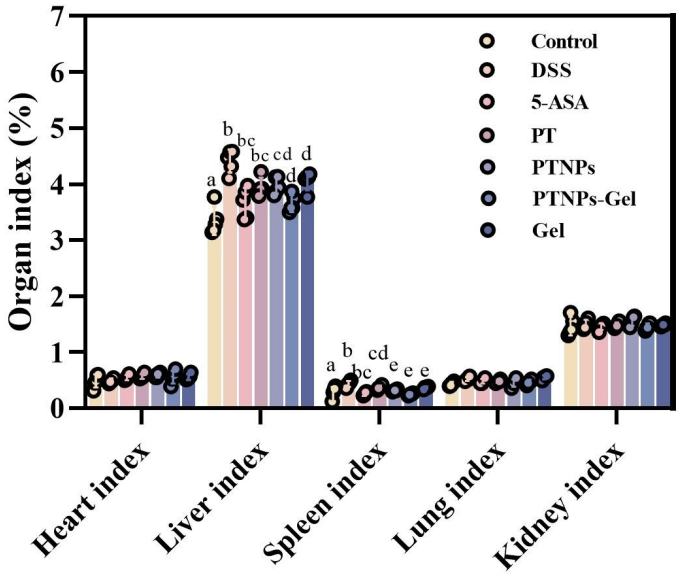


Fig. S8 Colon index of UC mice after different sample treatments. The values are mean ±SD (n = 5). * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001.



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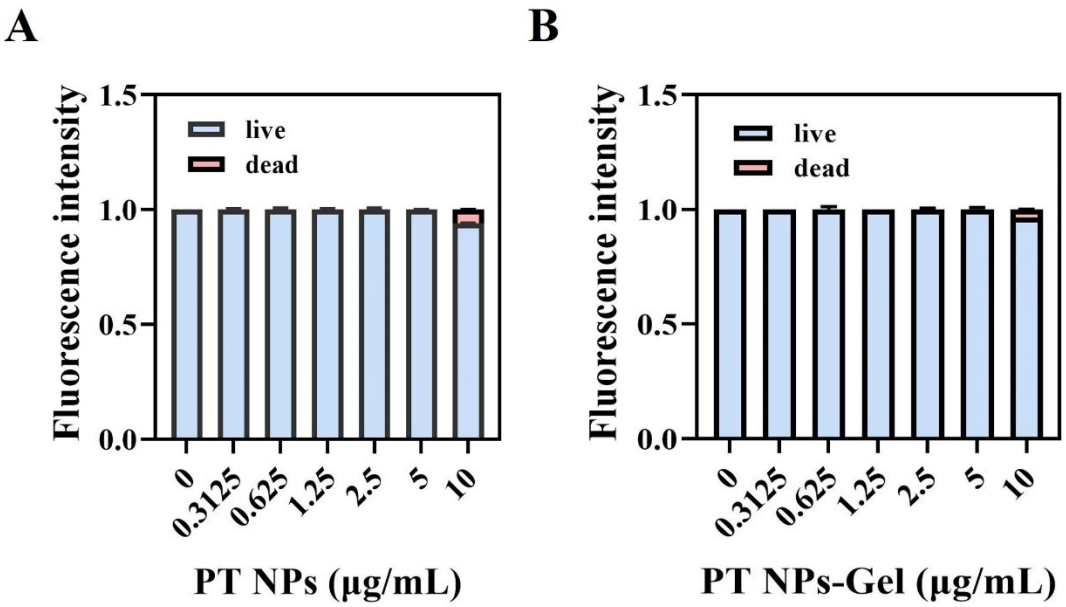
101 Fig. S9 Organ index of UC mice after different sample treatments. The values are mean ±SD (n =
102 5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.



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104 Fig. S10 In live/dead staining assay, the quantification of fluorescence intensity of (A) PT NPs and
105 (B) PT NPs-Gel groups was analyzed by Image J software. The data are expressed as means ± SD
106 (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

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