Supplementary Information

Targeted delivery and ROS-responsive release of celastrol by macrophage membrane biomimetic liposome alleviates acute kidney injury

Guangqin Cheng[a,&], Dongxu Bai[a,&], Weiyi Chen[a,&], Junmei Wang[a], Jian Xu[a], Yongping Zhang[a], Lin Li[d], Qing Lin[d], Zhirong Zhang[d], Jinghua Ruan[c,*], Ling Zhang[b,*], Ling Guo[a,*]

[a]National Engineering Technology Research Center for Miao Medicine,Guizhou Engineering Technology Research Center for Processing and Preparation of Traditional Chinese Medicine and Ethnic Medicine,College of Pharmaceutical Sciences, Guizhou University of Traditional Chinese Medicine, Guiyang, 550025, P. R. China.

[b]College of Polymer Science and Engineering, West China School of Public Health, Med-X center of materials, Sichuan University, Chengdu, 610065, P. R. China.

[c]The First Affiliated Hospital, Guizhou University of Traditional Chinese Medicine, Guiyang 550001, P. R. China.

[d]West China School of Pharmacy, Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry, Sichuan Engineering Laboratory for Plant-Sourced Drug and Sichuan Research Center for Drug Precision Industrial Technology, Sichuan University, Chengdu, 610041, P. R. China.

&These authors contributed equally: Guangqin Cheng, Dongxu Bai, Weiyi Chen

*Corresponding authors. E-mail address: RJH7737@163.com (Jinghua Ruan); zhangling83@scu.edu.cn (Ling Zhang); guoling032@gzy.edu.cn (Ling Guo).

Supplemental Figures.

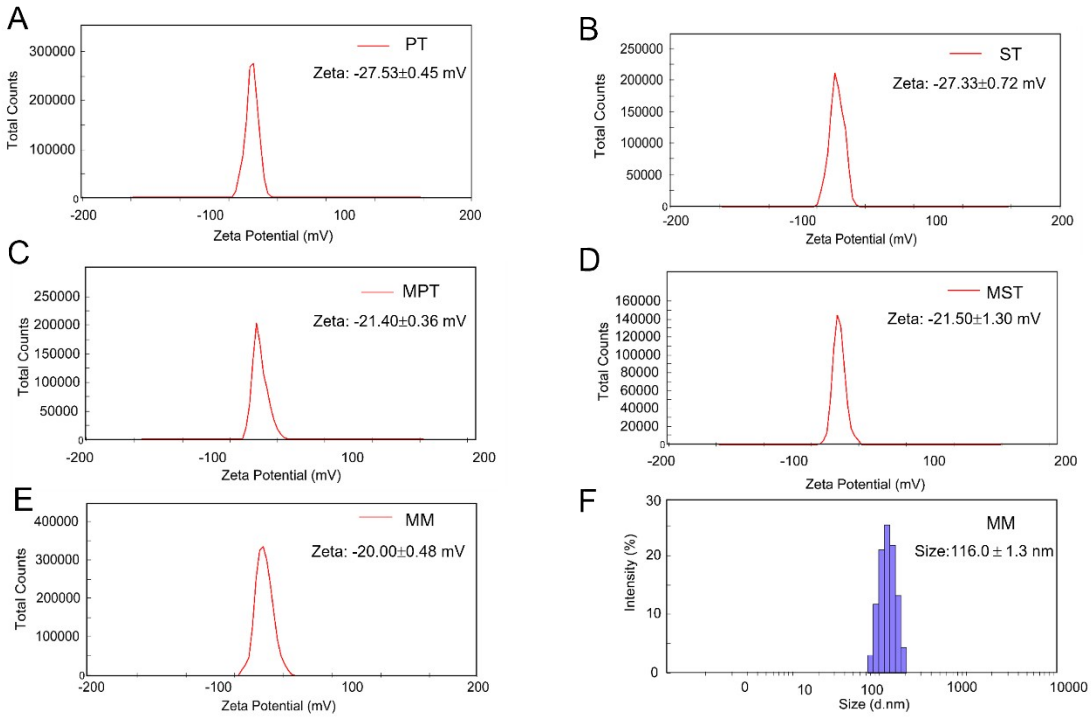

Figure S1. Characterization of MM and CLT formulations. (A-E) Zeta potential of PT, ST, MPT, MST, and MM measured by dynamic light scattering. (F) Particle size and size distribution of MM measured by dynamic light scattering. Data are mean ± S.D. (n = 3).

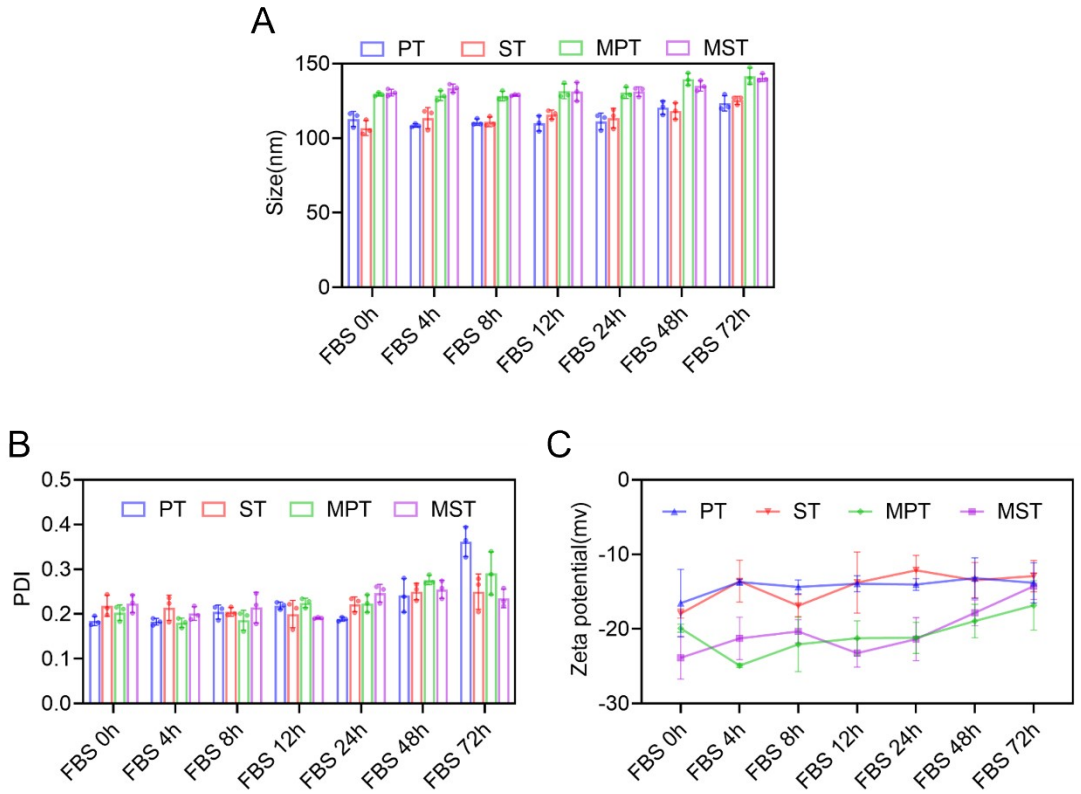

Figure S2. The serum stability of CLT formulations. (A-C) The variations in particle size, zeta potential, and PDI of PT, ST, MPT or MST after incubation with an equal volume (v/v) of FBS for 72 h. Data are mean ± S.D. (n = 3).

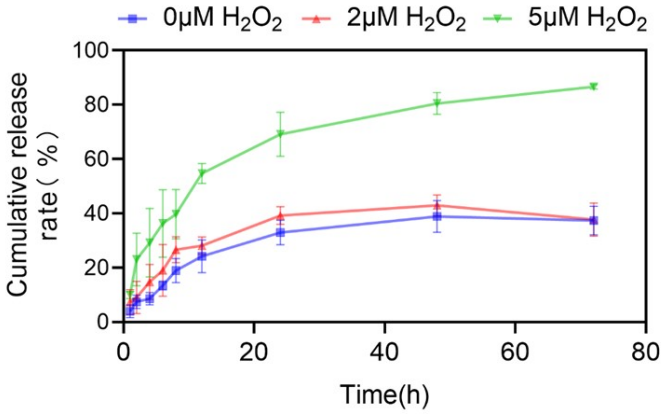

Figure S3. *In vitro* release profile of ST were evaluated in the presence of 0, 2, and 5 μM H_2O_2. Data are mean ± S.D. (n = 3).

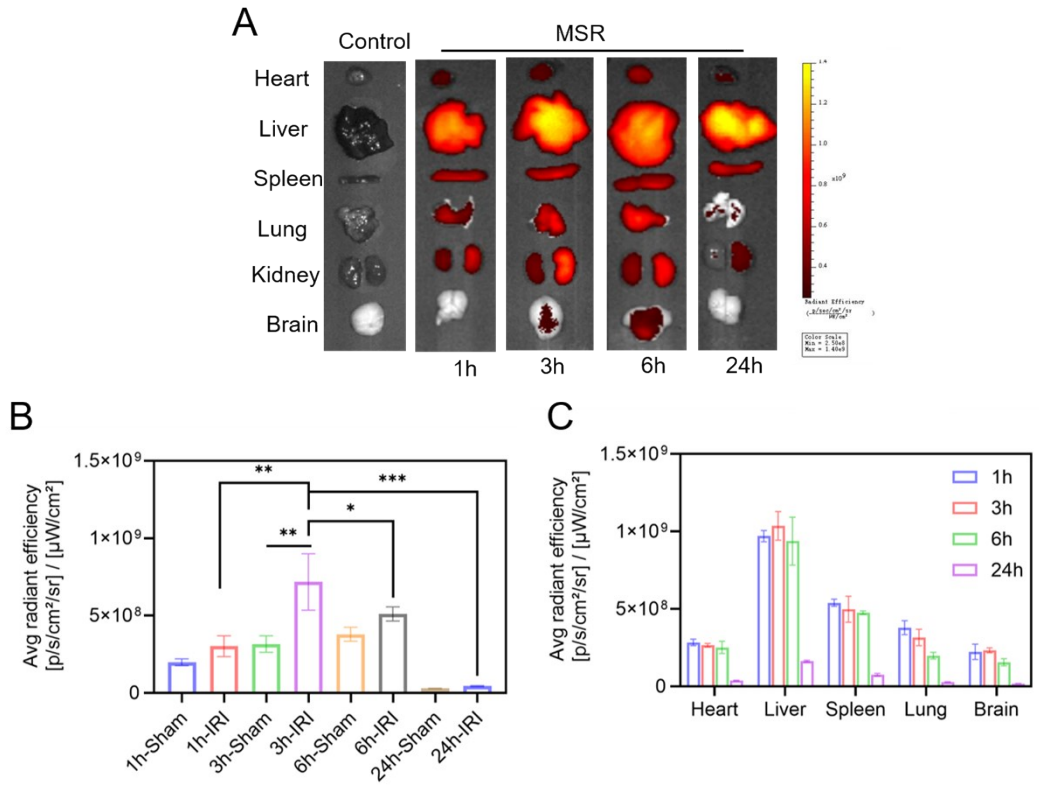

Figure S4. *In vivo* biodistribution of MSR in unilateral IRI mice. (A-C) The biodistribution and quantification of MSR accumulated in major organs were assessed at 1, 3, 6, and 24 h following intravenous administration. Data are mean ± S.D. (n = 3). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Statistical significance was determined by one-way ANOVA with Tukey *post hoc* test.

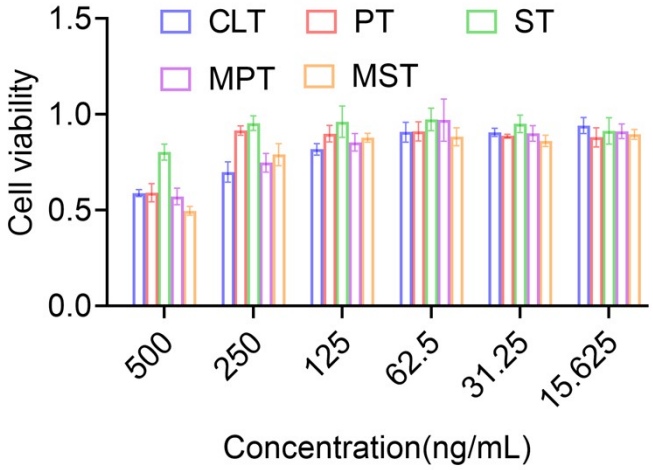

Figure S5. The cytotoxicity of CLT formulations on HK-2 cells. The HK-2 cell viability after incubating with various concentration of CLT, PT, ST, MPT, or MST for 24 h evaluated using the CCK-8 assay. Data are mean ± S.D. (n = 5).

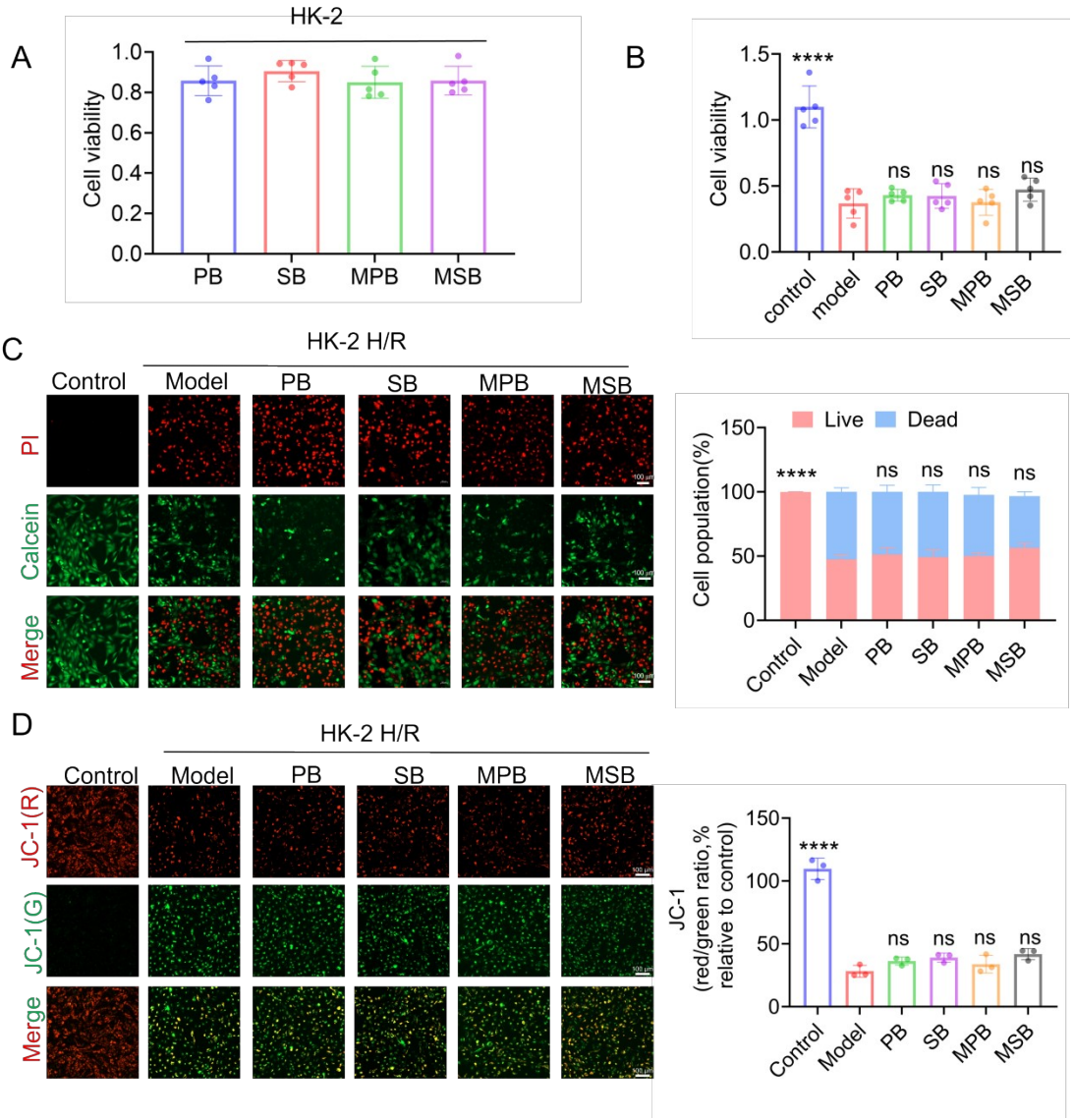

Figure S6. *In vitro* cytoprotective activities of PB, SB, MPB, and MSB. (A) The HK-2 cell viability after incubating with PB, SB, MPB, and MSB for 24 h evaluated using the CCK-8 assay (n = 5). (B) Cell viability of H/R treated HK-2 cells incubated with blank formulations (n = 5). (C) CLSM images and the semi-quantitative analysis of live and dead cells in H/R treated HK-2 cells stained with Calcein-AM/PI following various treatments (n = 3). Scale bar = 100 μm. (D) CLSM images and the semi-quantitative analysis of mitochondrial membrane potential in H/R treated HK-2 cells stained with JC-1 following various treatments (n = 3). Scale bar = 100 μm. Data are mean ± S.D., compared to the model group, **** $P < 0.0001$. ns, not significant. Statistical significance was determined by one-way ANOVA with Tukey *post hoc* test.

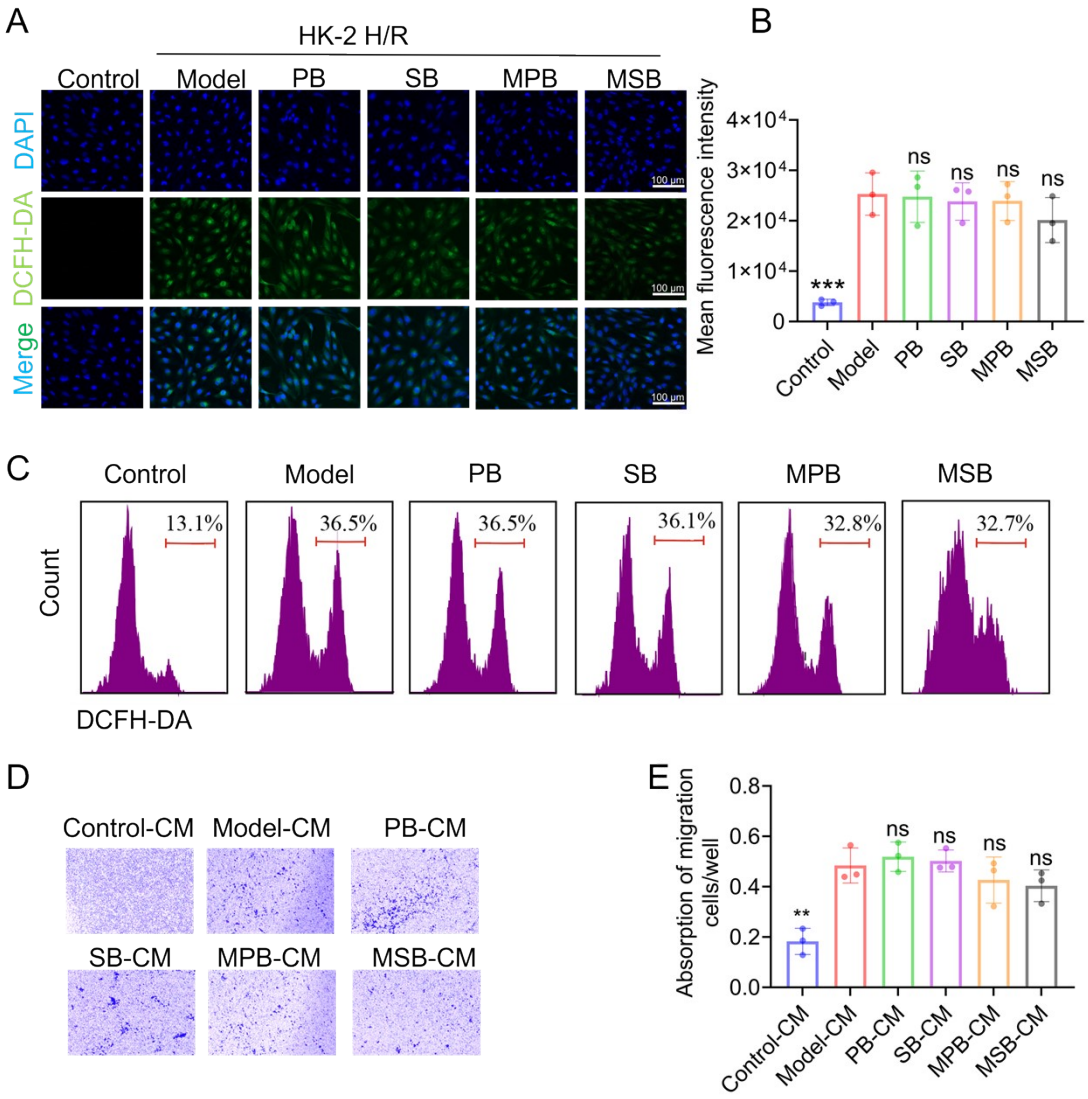

Figure S7. *In vitro* anti-oxidant and anti-inflammatory activities of PB, SB, MPB, and MSB. (A) CLSM images of intracellular ROS in H/R treated HK-2 cells following various treatments. Scale bar = 100 μm. (B) quantitative results and (C) FACS analysis of ROS level in H/R treated HK-2 cells following various treatments (n = 3). (D) Representative images of migrated THP-1 macrophages subjected to various CM. (E) Semi-quantitative analysis of macrophage migration (n = 3). Data are mean ± S.D., compared to the model group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, not significant. Statistical significance was determined by one-way ANOVA with Tukey *post hoc* test.

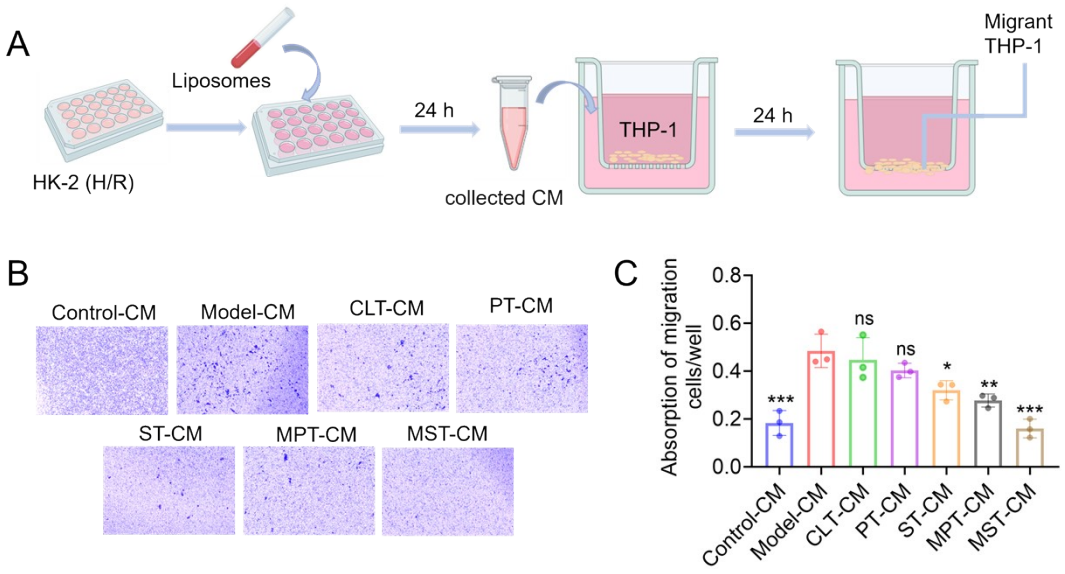

Figure S8. Evaluation of the anti-inflammatory effect of MST *in vitro*. (A) Schematic illustration of the experimental procedure for collecting CM and conducting the Transwell migration assay. (B) Representative images of migrated THP-1 macrophages subjected to various CM. (C) Semi-quantitative analysis of macrophage migration. Data are mean ± S.D. (n = 3). Compared to the model-CM group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, not significant. Statistical significance was determined by one-way ANOVA with Tukey *post hoc* test.

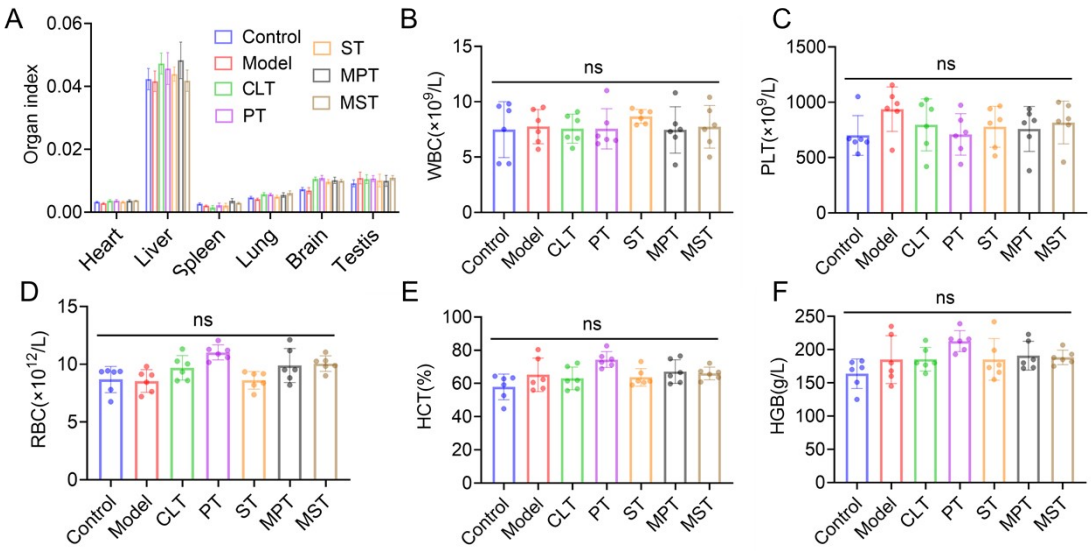

Figure S9. The biosafety evaluation of CLT formulations. (A) The changes in the organ index of following intravenous administration of saline, CLT, PT, ST, MPT or MST. (B–F) WBC, PLT, RBC, HCT and HGB levels in blood of rats following intravenous administration of saline, CLT, PT, ST, MPT or MST. Data are mean ± S.D. (n = 6). ns, not significant. Statistical significance was determined by one-way ANOVA with Tukey *post hoc* test.