Supplementary Information

Construction of sustained release hydrogel using gallic acid and lysozyme with antimicrobial properties for wound treatment

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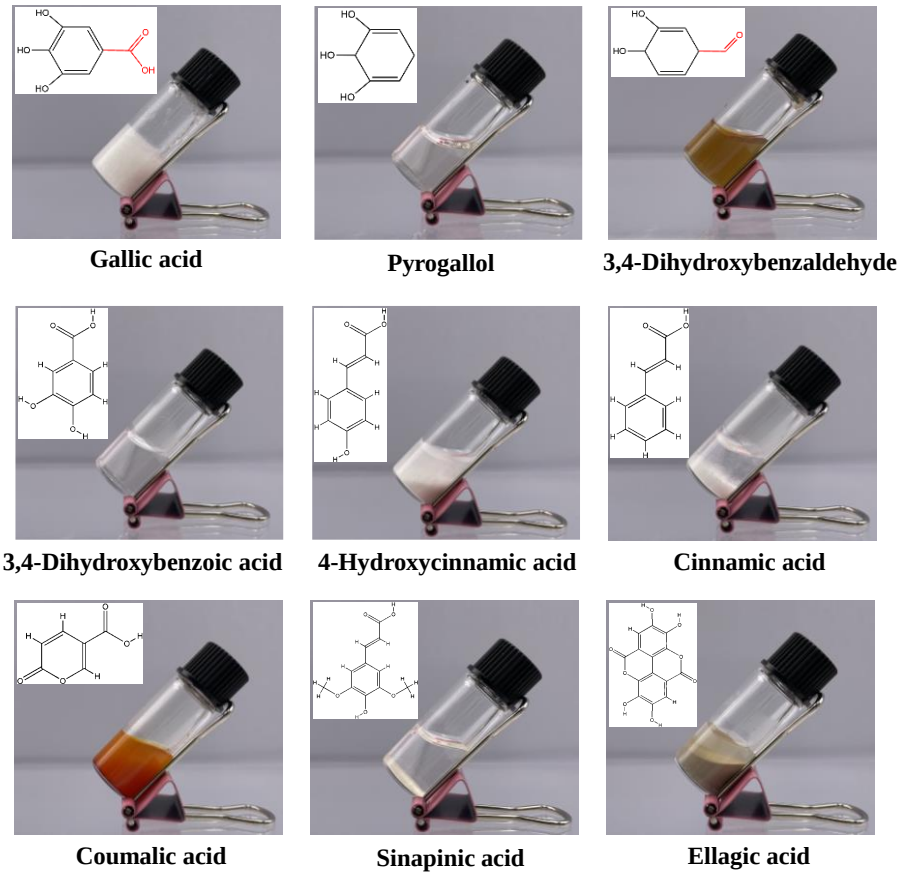
Table S1. Sequence of Primers for q-PCR

Target gene	Primer sequence
GADPH-F	AACTTTGGCATTGTGGAAGG
GADPH-R	ACACATTGGGGGTAGGAACA
IL-6-F	TGGAAATGAGAAAAGAGTTGTGC
IL-6-R	CCAGTTTGGTAGCATCCATCA
TNF-α-F	ATCTACCTGGGAGGCGTCTT
TNF-α-R	GAGTGGCACAAGGAACTGGT
IL-1β-F	TTCATCTTTGAAGAAGAGCCCAT
IL-1β-R	TCGGAGCCTGTAGTGCAGTT
TGF-β-F	TGGAGCAACATGTGGAACTC
TGF-β-R	TGCCGTACAACTCCAGTGAC
NLRP3-F	ATCAACAGGCGAGACCTCTG
NLRP3-R	GTCCTCCTGGCATACCATAGA
HO-1-F	CACGCATATACCCGCTACCT
HO-1-R	CCAGAGTGTTCATTCGAGCA
Nrf2-F	CTTCCATTTACGGAGACCCAC
Nrf2-R	GATTCACGCATAGGAGCACTG
iNOS-F	TTTCCTGTGCTGTGCTACAGTT
iNOS-R	CCACTCGTATTTGGGATGCT
COX2-F	GGTGCCTGGTCTGATGATGTATGC
COX2-R	GGATGCTCCTGCTTGAGTATGTCG
MCP-1-F	CCACTCACCTGCTGCTACTCATTC
MCP-1-R	CTTCTTTGGGACACCTGCTGCTG
TLR4-F	CCGCTTTCACCTCTGCCTTCAC
TLR4-R	ACCACAATAACCTTCCGGCTCTTG
STAT3-F	AATCTCAACTTCAGACCCGCCAAC
STAT3-R	GCTCCACGATCCTCTCCTCCAG
Claudin-1-F	AGTGCATGAGGTGCCTGGAAG
Claudin-1-R	TGGCCACTAATGTCGCCAGA
Occludin-F	GGCAAGCGATCATACCAGAG
Occludin-R	AGGCTGCCTGAAGTCATCCAC

ZO-1-F	GACCAATAGCTGATGTTGCCAGAG
ZO-1-R	TATGAAGGCGAATGATGCCAGA

A



B

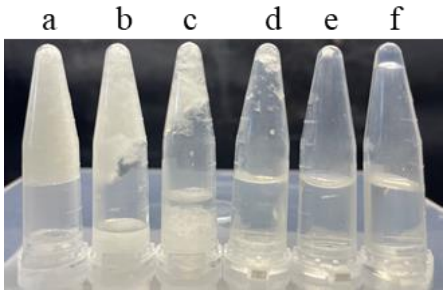


Fig. S1. (A) Schematic diagram of different small molecule acids (60mg/ml) after co-incubation with lysozyme (1mg/ml)**. (B)** Different ratio between gallic acid and lysozyme more detail. Where a represents the ratio of gallic acid to lysozyme is 60:1, b represents 55:1, c represents 55:1.5, d represents 55:2, e represents 50:1, f represents 40:1, respectively.

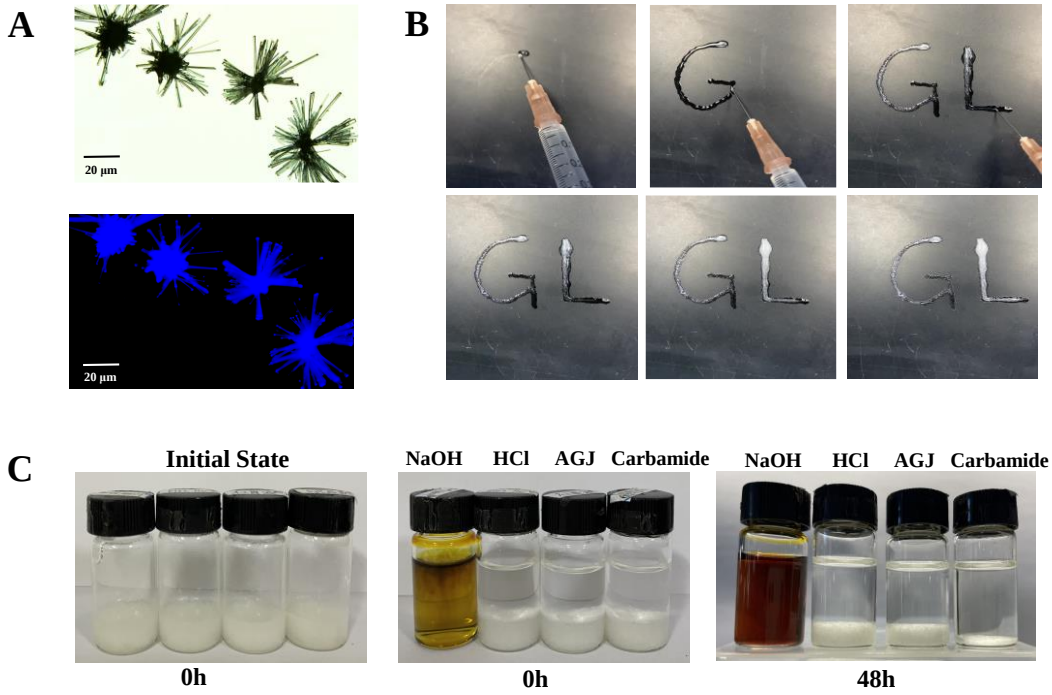


Fig. S2. (A) Inverted fluorescence microscope images showing blue fluorescent fiber of GL hydrogel. **(B)** Injection behavior and in situ formation of GL hydrogel. **(C)** The stability of GL hydrogel in acidic (0.01mol/L HCl), alkaline (1mol/L NaOH), artificial gastric juice (AGJ) and 50 % carbamide solution.

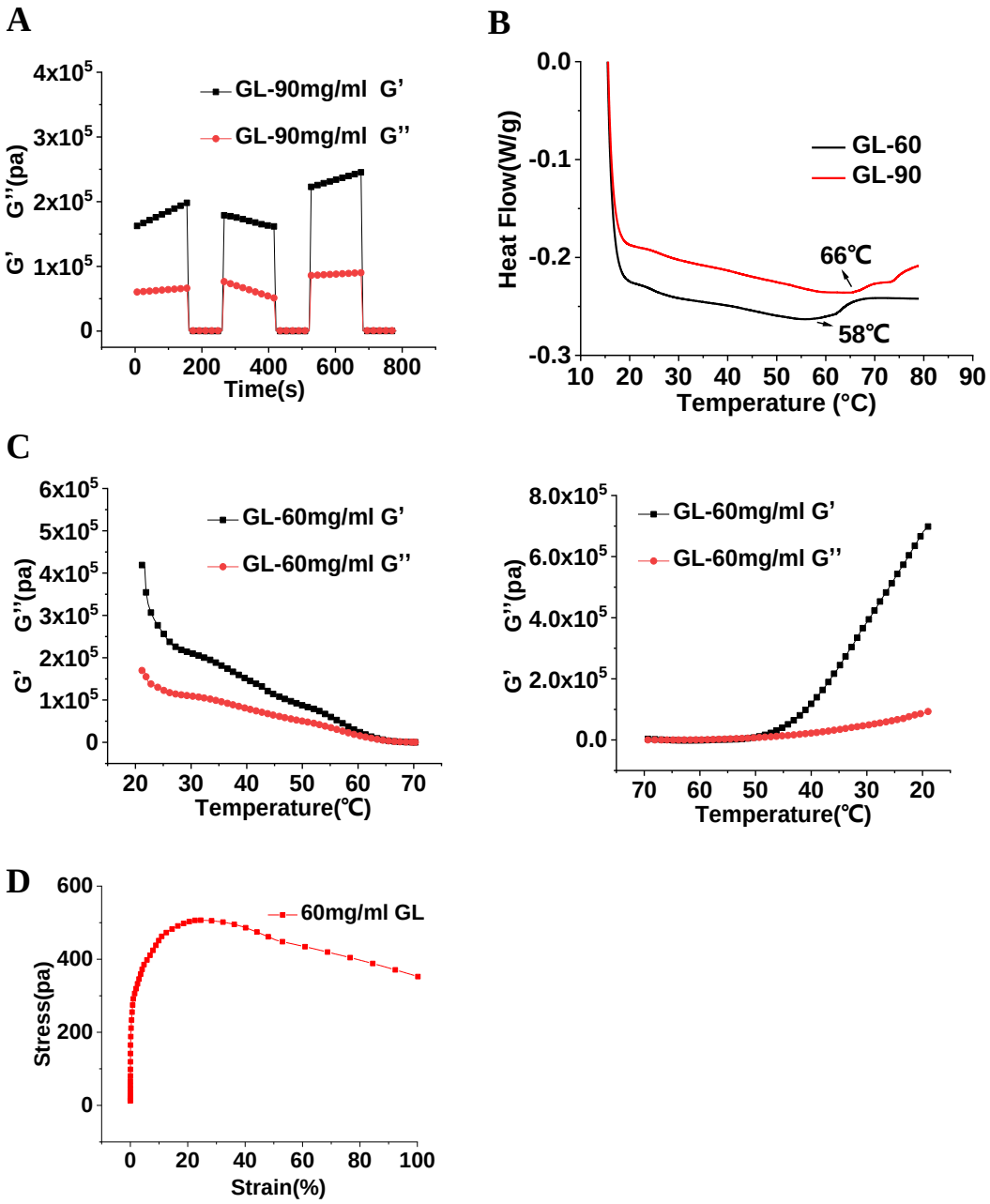


Fig. S3. (**A)** Step-strain measurements of the hydrogel three cycles at low strain (0.01%) and high strain (10%), frequency 10 Hz. **(B)** DSC thermogram of the GL hydrogel with the concentration of 60 mg/ml and 90 mg/ml (heating rate of dT / dt = 5 ℃/min, 10 µl aluminum crucibles under N_2 atmosphere). **(C)** The responsiveness in environment of GL hydrogel at different temperatures, fixed frequency (10 Hz) and strain (0.01%). **(D)** The curve of stress and strain under 0.1 s^{-1} shear rate. The rheological measurement mode is stress growth.

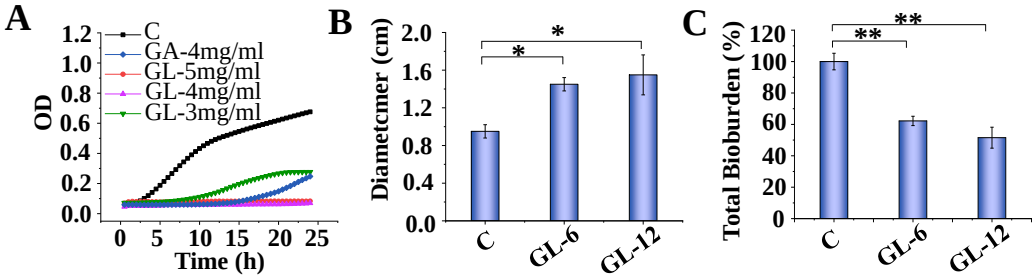


Fig. S4. *In vitro* antibacterial activities of the GL hydrogel against *S. aureus*. (**A)** Kinetics of the inhibition of bacterial growth. The effect of the GL hydrogel and controls on bacterial growth was evaluated by turbidity analysis via absorbance readings at 600 nm of bacteria treated overnight under 30 ℃. **(B)** Bacteriostatic zone diameter of bacteria treated with GL hydrogel of 0 mg/ml (C), 6mg/ml (GL-6), 12mg/ml (GL-12), respectively. **(C)** Antibacterial biofilm effect of GL hydrogel of 0 mg/ml (C), 6mg/ml (GL-6), 12mg/ml (GL-12), respectively.

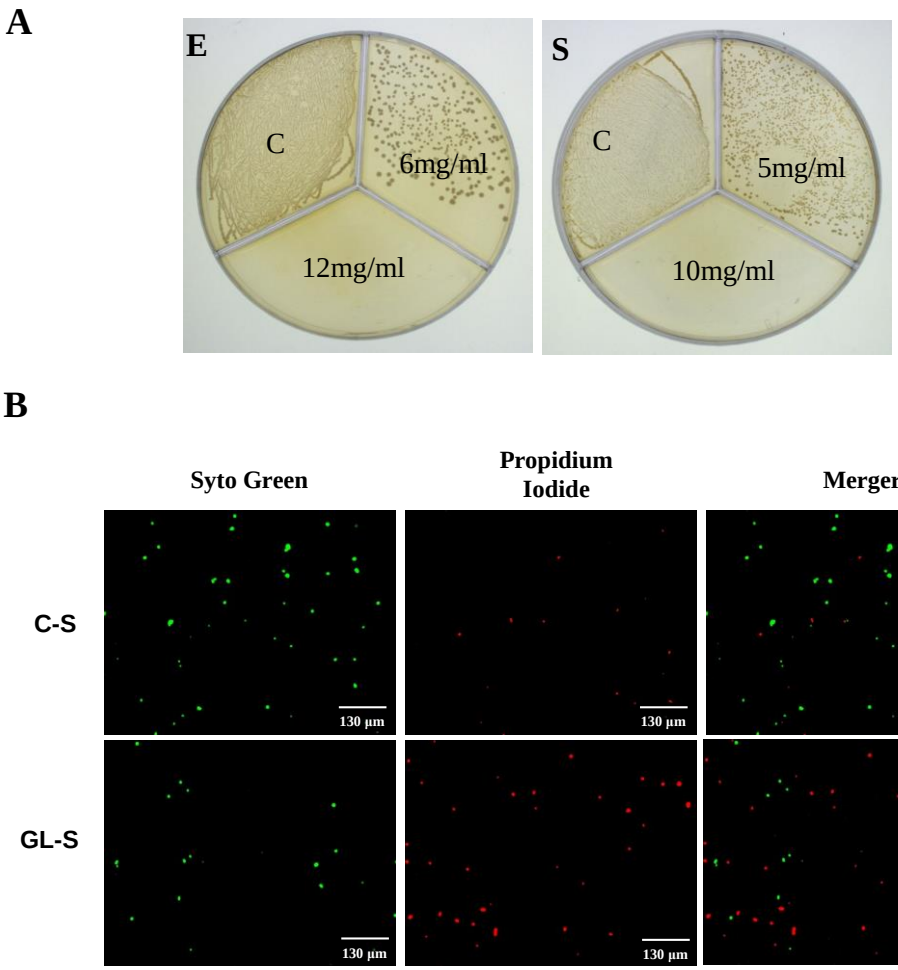


Fig. S5. (A) Minimum bactericidal concentration of *E. coli* (E) and *S. aureus* (S) treated with different concentrations of GL hydrogel. **(B)** Live/dead bacterial viability assay in *S. aureus* treated with GL hydrogel. Bacterial cells with intact membrane are stained green, and bacteria with compromised membrane are stained red.

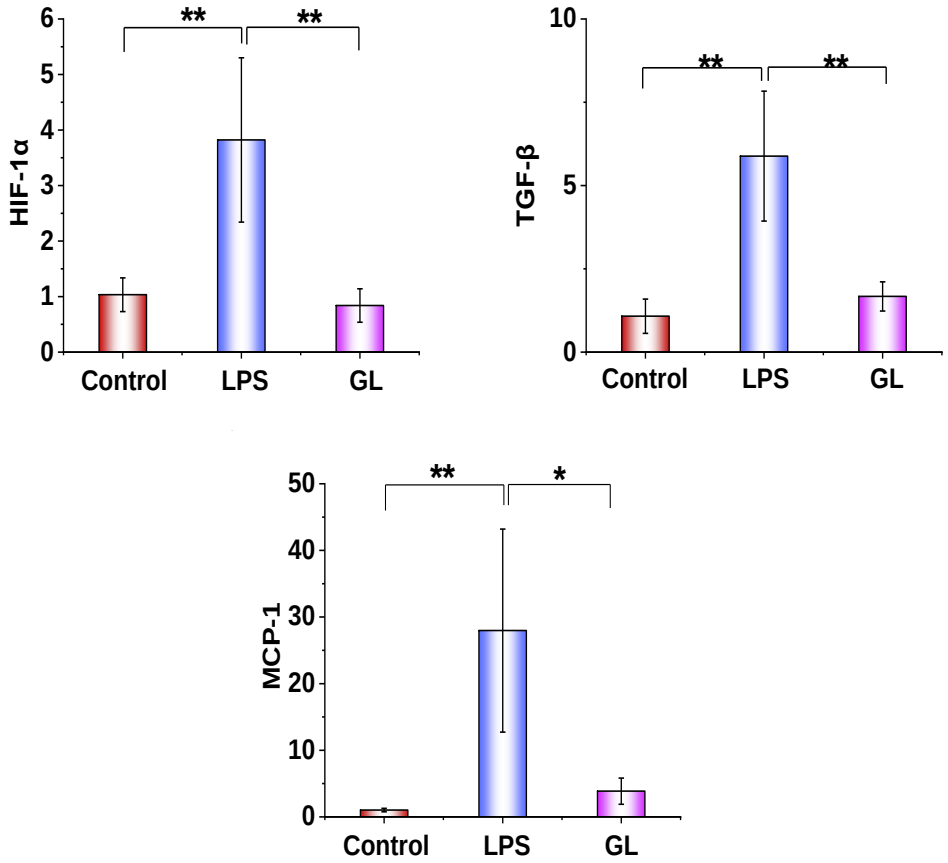


Fig. S6. Expression levels of HIF-1α, TGF-β and MCP-1 in RAW 264.7 cells after treatment with LPS and GL hydrogel. Used q-PCR method and normalized using GADPH as the housekeeping gene.

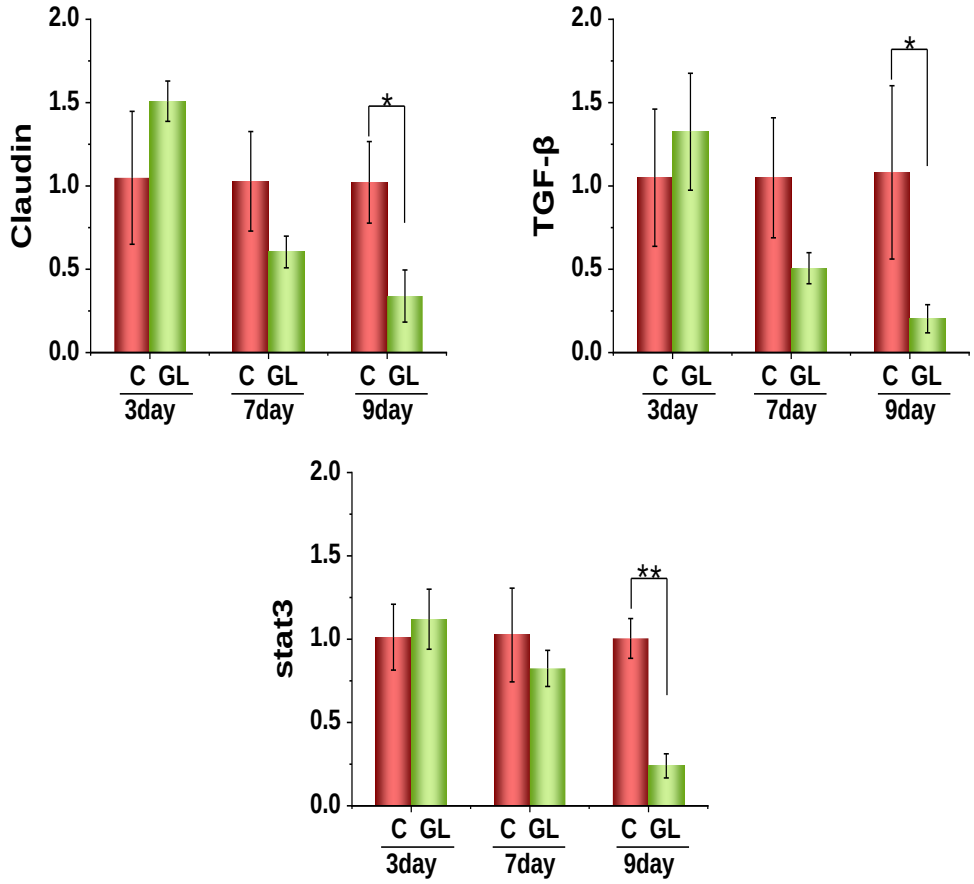


Fig. S7. Expression levels of Claudin, TGF-β and stat3 in skin tissues from control or GL hydrogel groups on days 3, 7 and 9. Used q-PCR method and normalized using GADPH as the housekeeping gene.

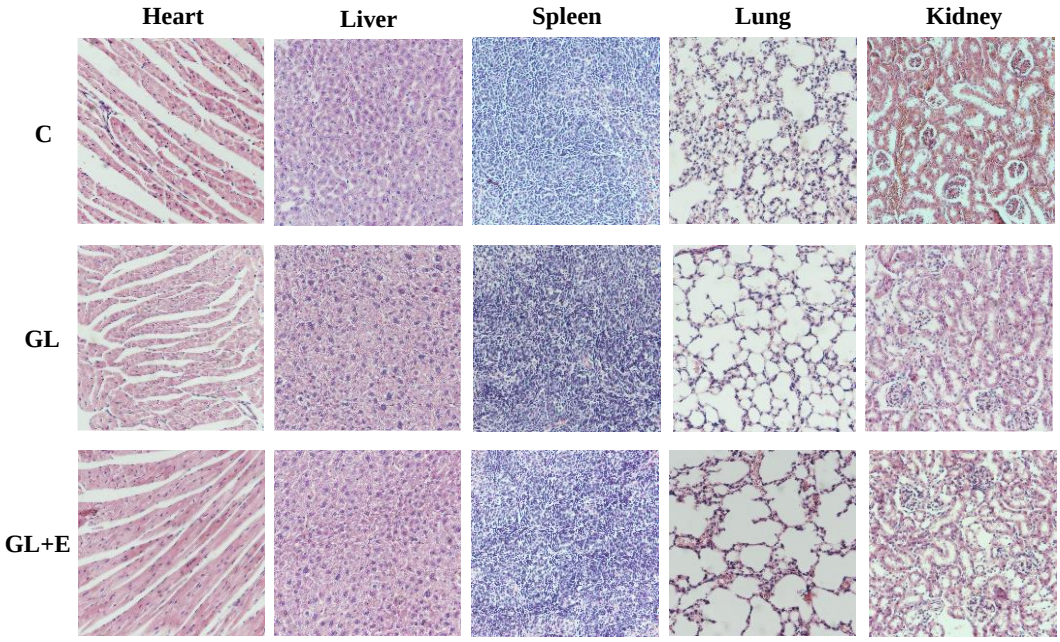


Fig. S8. Micrographs of H & E stain major organ tissue slices from different groups after 9d of treatment.