Supporting Information

Title : Targeted treatment of glycerol-induced AKI in rats using BMSCs derived magnetic exosomes and its mechanism

Author:

Yuling Chen

Institute of Disaster and Emergency Medicine, Tianjin University, Tianjin, China.

Tianjin Key Laboratory of Disaster Medicine Technology, Tianjin, China.

Shike Hou

Institute of Disaster and Emergency Medicine, Tianjin University, Tianjin, China.

Tianjin Key Laboratory of Disaster Medicine Technology, Tianjin, China.

Corresponding Author: Yuling Chen

E-mail addresses: chenyuling97@outlook.com

Contents

Supplementary

Materials and Methods

Prussian blue staining

Make cell slides. Cells were fixed in polyformaldehyde solution at room temperature for 30 minutes and washed twice with PBS. The cells were incubated in a solution of 1% hydrochloric acid and 2% potassium ferrocyanide for 40 minutes, washed twice with distilled water, and re stained with nuclear fixation red for 15 minutes. Then clean the cells twice with distilled water. Observation under an optical microscope: Iron nanoparticles appear as blue spots inside the cell, while the nucleus appears red.

TEM

The ultrastructural morphology of cells and exosomes was observed by transmission electron microscopy.

Exosomes were fixed in Sorensen buffer (pH 7.4) with 2% glutaraldehyde for 2 hours, while exosomes contained in agarose gel were fixed in 0.1 M phosphate buffer with 2.5% glutaraldehyde and 2% paraformaldehyde. All samples were fixed in 1% osmium tetroxide for 2 hours before being cut. Then dehydrate the slices at a graded concentration of acetone and embed them into the Epon Araldite mixture. Inspect the semi thin section (with a thickness of 1 μm) using an optical microscope and stain with toluidine blue. Cut the ultra-thin section into 70 nm thick sections and place them on a Cu grid. Transmission electron microscopy images were collected using TEM at a voltage of 80 kV and equipped with a Megaview II camera for digital image acquisition.

Preparation of cell samples by treating BMSC with SPION 25 μg/mL. After 24 hours of incubation, the cells were separated and fixed at 2% glutaraldehyde solution for 2 hours at 4 ° C. Then, they were washed three times in buffer (pH 7.2) and treated at room temperature with 2.5% potassium ferrocyanide and 1% osmium tetroxide for 1 hour. After washing, cells are dehydrated with acetone with a 30% increase in concentration (×2) 50% (×2) 70% (×2) 90% (×2) Then further dehydrate with 100% acetone. After drying, the sample was embedded overnight in a solution of epoxy resin and acetone (100%) (50:50), and then embedded in 100% epoxy resin for 4-5 hours. The sample was dried at 60 ℃ for 2 days and then cut into thin slices of 70 nanometers. Use TEM to observe the internal situation of cells.

Results

Fig.S.1.

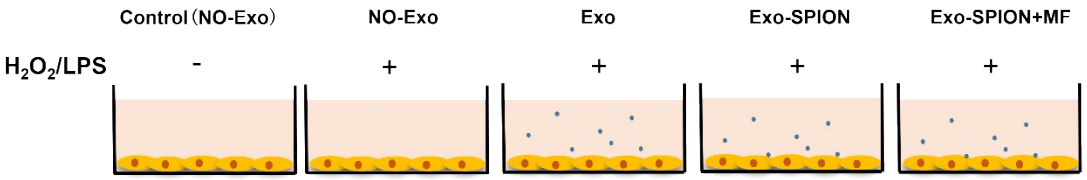


Fig.S.1. Cell grouping. Inoculate L6/NRK-52e in 6-well plate ($1x10^5$ cells/well) for 24 hours. After being stimulated for 10 minutes with H_2O_2 (200 μM) or LPS (100ng/mL) for 24 hours, the supernatant was replaced with the above conditioned medium and cultured at 37°C for 6 hours.

Fig.S.2.

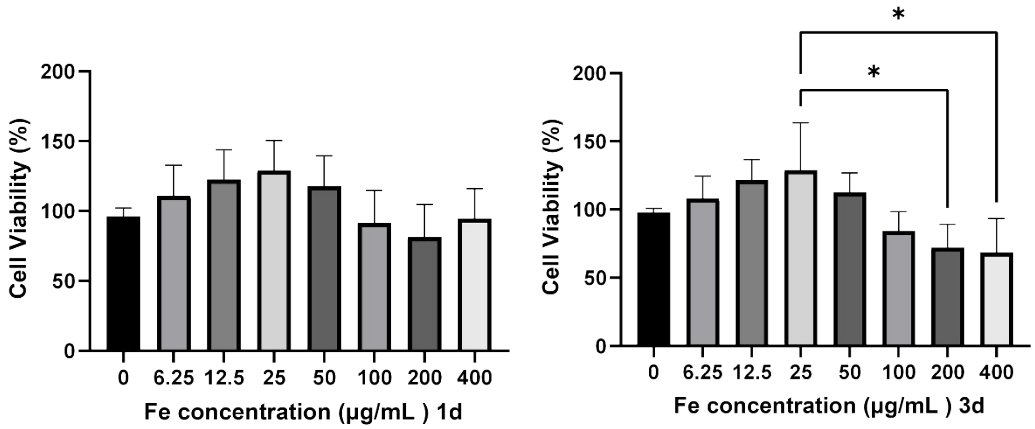


Fig.S.2. Toxicity study of the SPION on NRK-52e. CCK-8 assay was used to evaluate the proliferation of NRK-52e treated with different concentrations of SPION. (* *P<0.05,* n=6).

Fig.S.3.

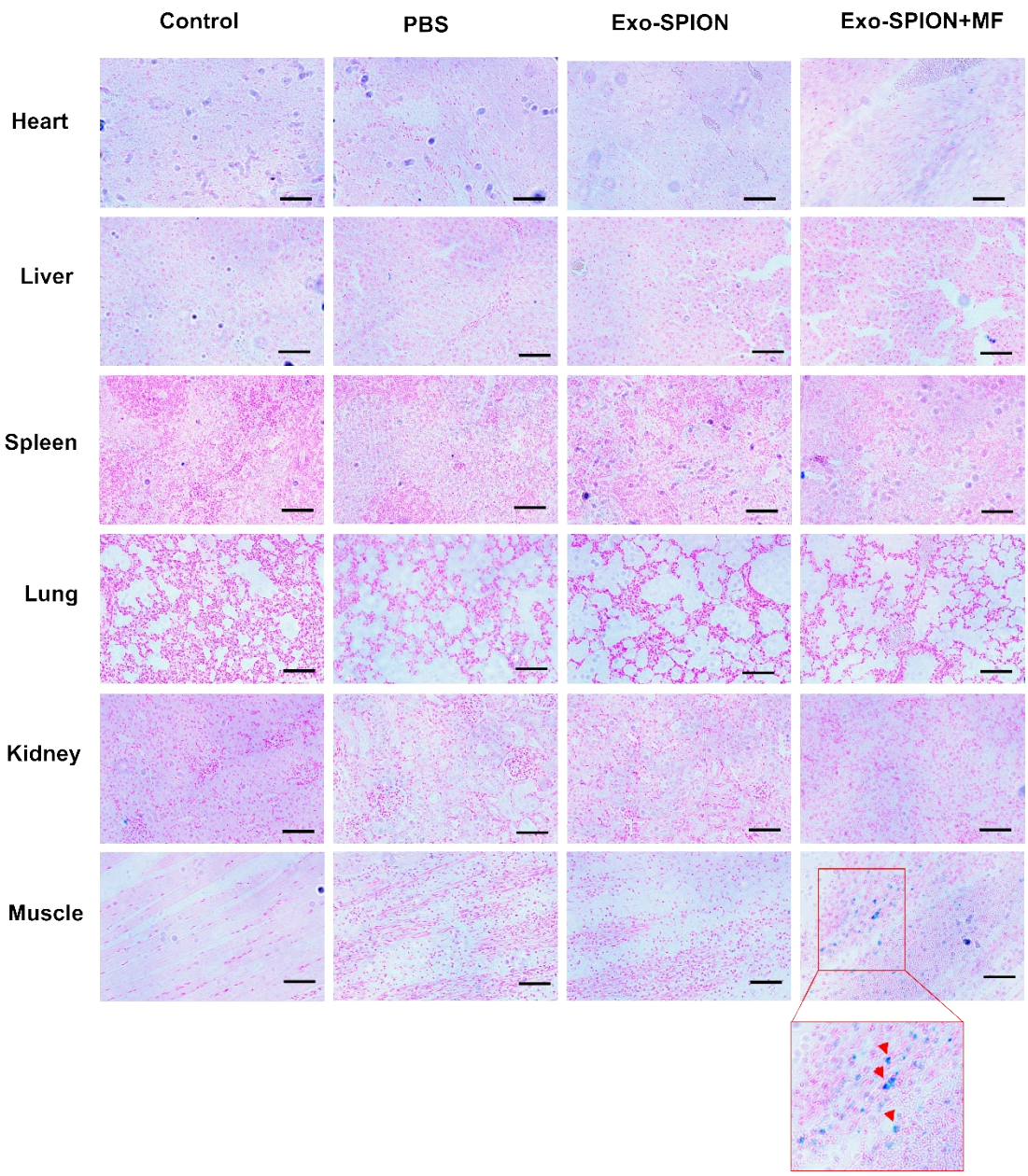


Fig.S.3. In vivo localization of Exo-SPION in rats AKI injury model under magnetic navigation. The representative micrograph of Prussian blue staining of paraffin sections of heart, liver, spleen, lung, kidney and gastrocnemius muscle on the third day after the tail vein injection of Exo-SPION in rats. The white arrow shows the positioning of the Exo-SPION. Scale bar, 10 μm.

Fig.S.4.

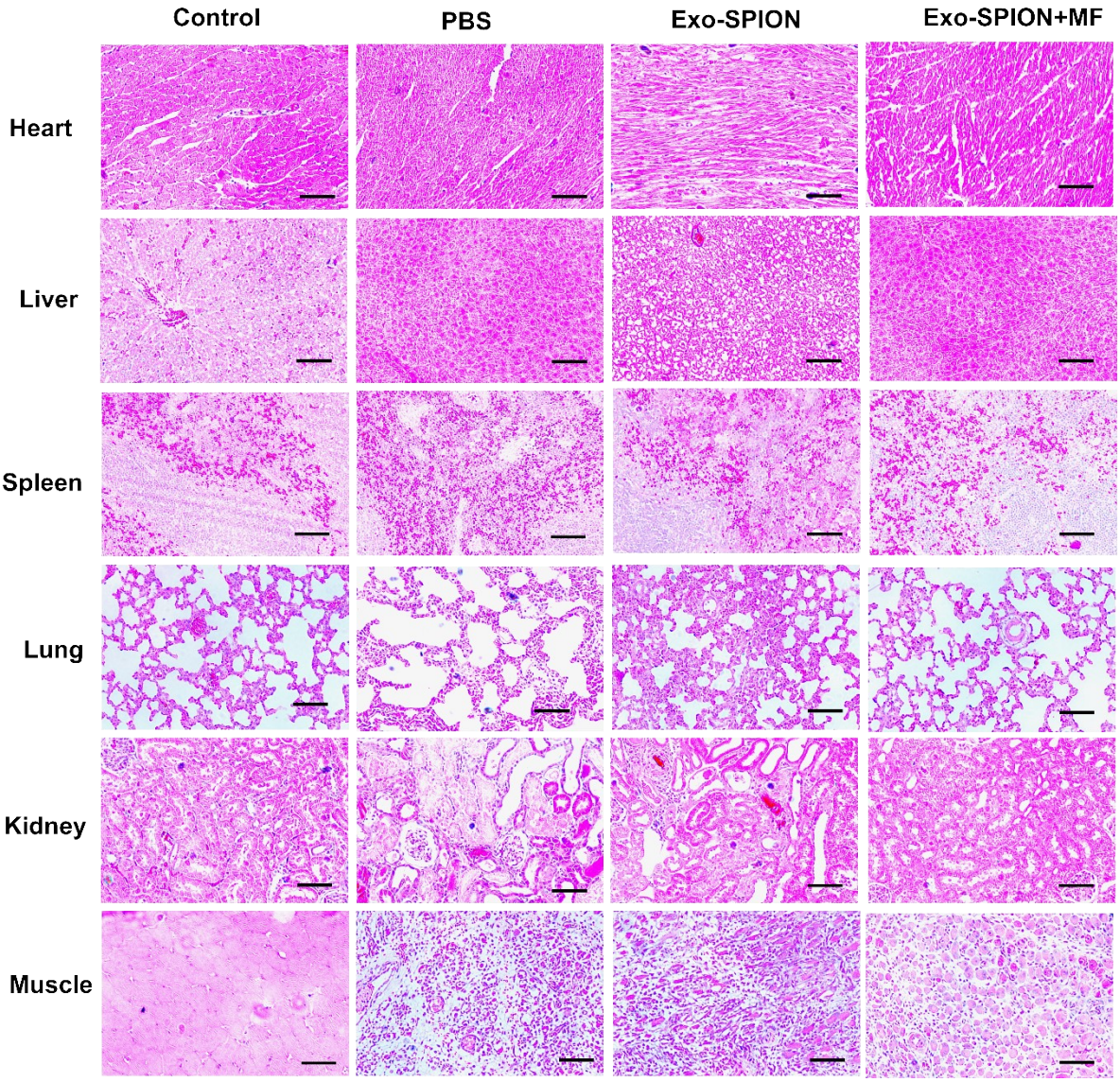


Fig.S.4. HE stains of organ toxicity in Exo-SPION. Evaluate pathological changes in heart, liver, spleen, lungs, kidneys and muscle tissues through section microscopy examination (HE stains); Scale bar, 100 μm. There were no significant changes in the heart, liver, spleen and lungs. The renal tubules in the PBS and Exo-SPION groups were severely damaged. The Exo-SPION+MF group showed significant improvement. Control muscle tissue structure is normal. The substantial destruction of muscle fiber structure and the absence of inflammatory infiltration in the model group. The PBS group and Exo-SPION+MF group showed significant muscle edema, muscle fiber degeneration and atrophy.

Fig.S.5.

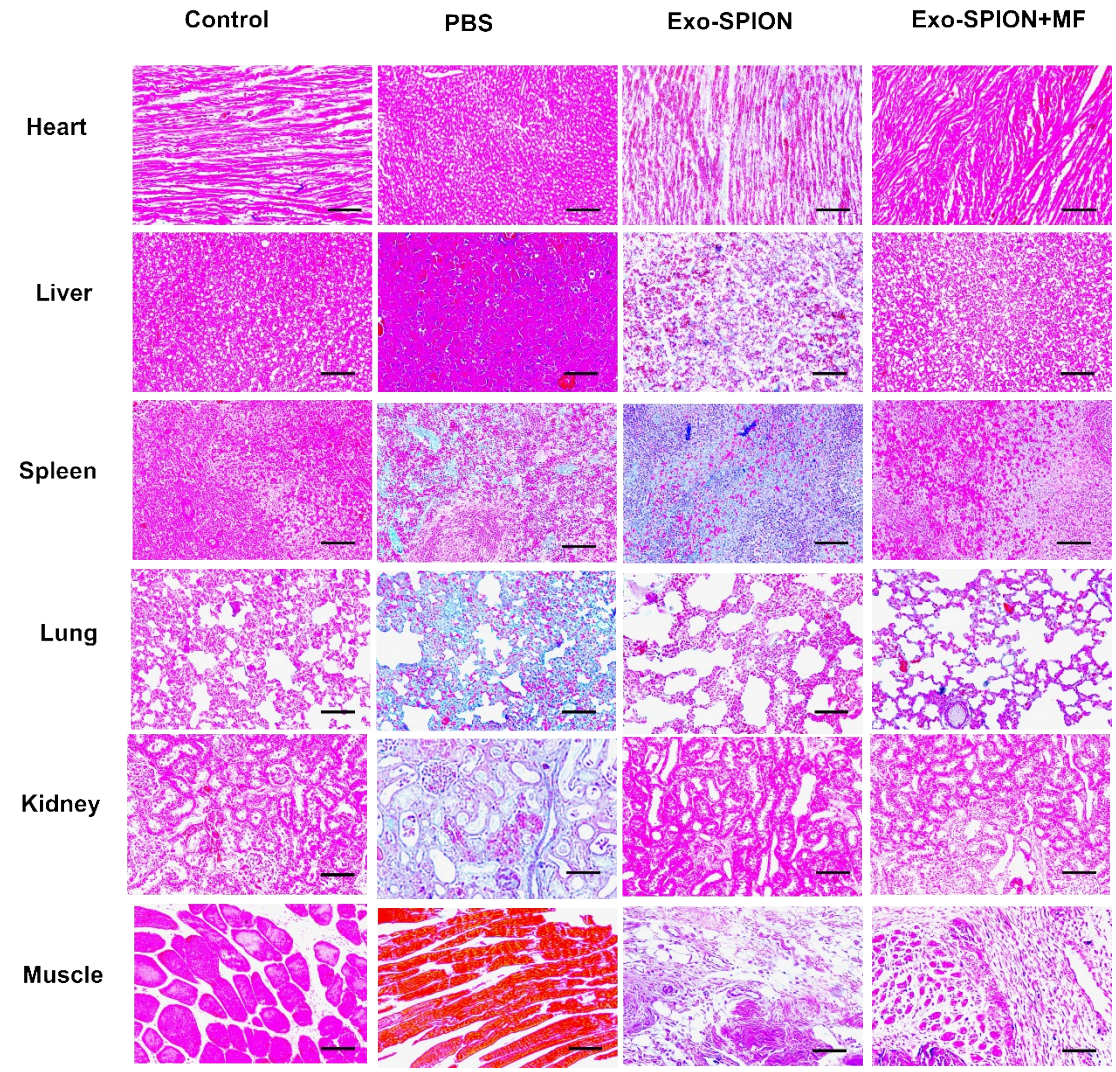


Fig.S.5. Masson stains of organ toxicity in Exo-SPION. Evaluate pathological changes in heart, liver, spleen, lungs, kidneys and muscle tissues through section microscopy examination; Scale bar, 100 μm.

Fig.S.6.

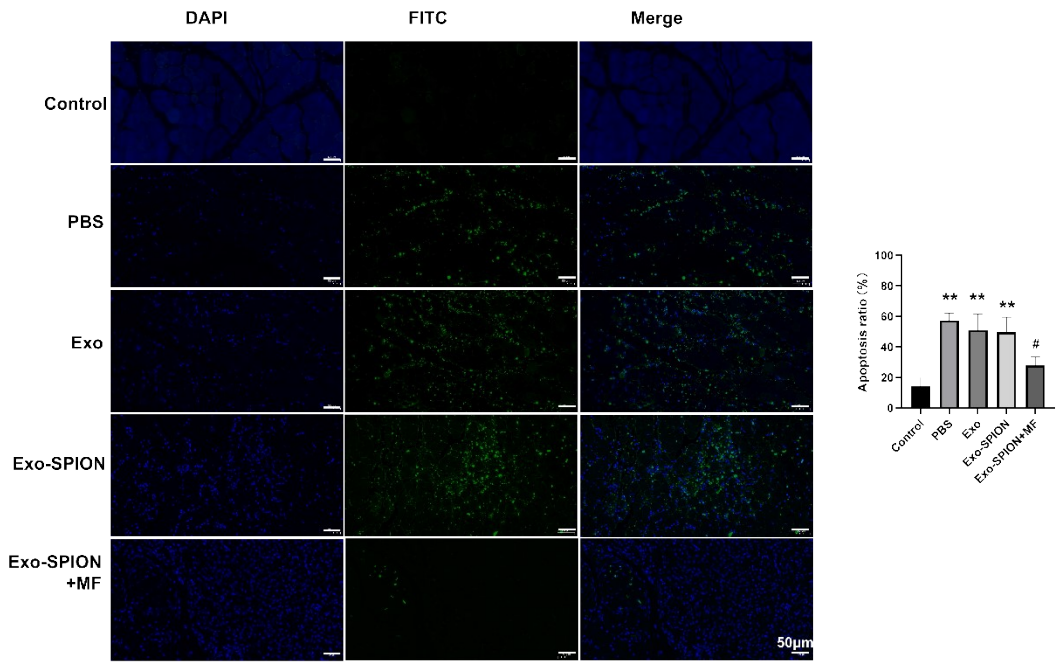


Fig.S.6. Percentage of apoptotic positive cells in muscle tissue of rats in each group. ** *P<0.01* compared to the Control group. # *P<0.05* compared to PBS group.

Fig.S.7.

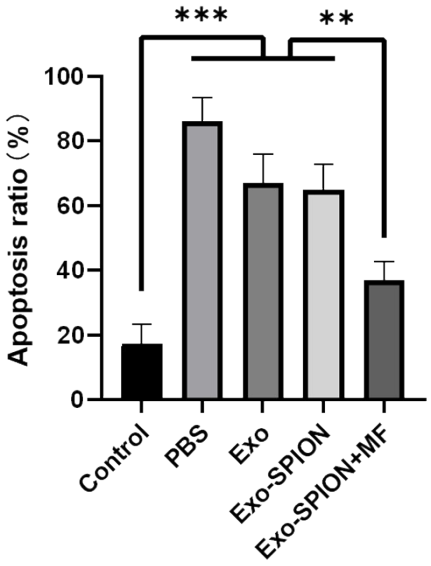


Fig.S.7. The percentage of apoptotic positive cells in renal tissue of rats in each group. ** *P<0.01,* ****P<0.001.*

Fig.S.8.

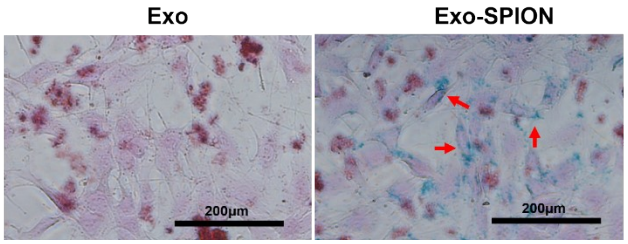


Fig.S.8. Exo-SPION internalized by L6 cells. Blue represents the SPION in the cell (red arrow). Scale bar, 200μm.

To verify the results of in vitro modeling, cells were stimulated with inducer H_2O_2 (200 μM) for 10 minutes and treated with LPS (100 ng/mL) for 24 hours. The viability of L6 cells was detected using CCK-8. The CCK results showed (Fig.S.9) that the cell viability of the H_2O_2 modeling group was significantly reduced compared to the control group ($P < 0.01$). At the same time, the cell viability of the LPS modeling group significantly decreased compared to the control group ($P < 0.05$), indicating the success of in vitro modeling.

Fig.S.9.

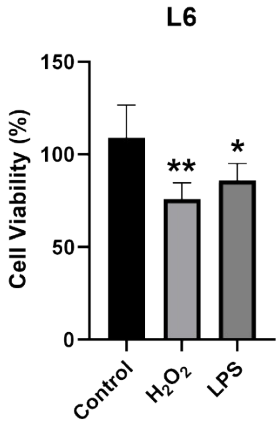
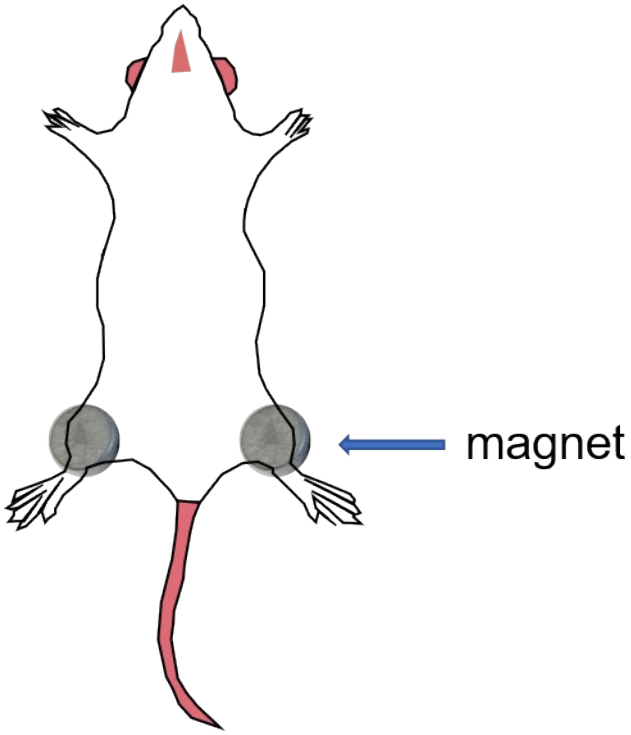


Fig.S.9. CCK detection of the effects of H_2O_2 and LPS stimulation on L6 cells. * *P<0.05,* ***P<0.01* relative to the Control group.

117 **Fig.S.10.**



118

119 Fig.S.10. Placement of the magnets during the treatment process.