

Supplementary file

## Methods

### Establishment of the collagenase-induced cerebral hemorrhage model

In order to induce cerebral hemorrhage, 1.2 µl of collagenase IV solution (0.2 U) was injected into the basal ganglia at a rate of 0.25 µl/min. A non-invasive blood pressure measurement system was used to measure blood pressure before and after the surgery. After the operation, the rats were resuscitated in the incubator and then returned to the cage. In the sham group, only needles were put in without collagenase injection.

The groupings are as follows: A, the sham group, in which the rats were given drinking water by gavage; B, the vehicle group, in which the rats were given drinking water by gavage; C, the drug administration group, in which the rats were given 10 mg/kg Sanguin by gavage. All groups were administered at 12 h, 36 h, and 60 h postoperatively.

MRI scan to assess the degree of cerebral edema.

12 h before the first administration and 72 h after cerebral hemorrhage, the experimental animals were scanned for their heads, and the scanned images were analyzed with Image J software. The volume of the affected hemisphere and contralateral hemisphere was calculated through the sum of the area of the affected hemisphere and contralateral hemisphere times the layer thickness. The brain swelling index was measured to assess the degree of brain edema. Brain swelling index (%) = (volume of the affected hemisphere – contralateral hemisphere)/volume of the contralateral hemisphere × 100%.

### Determination of brain water content

On the 3<sup>rd</sup> day after the operation, the rats were anesthetized, then quickly decapitated, and the brain tissues were stripped off. About 100 mg of brain tissue around the hematoma was cut out on a dry and clean table. At the same time, about 100 mg of brain tissue and cerebellum tissue in the contralateral hemisphere of the hematoma were obtained. The tissue was wet weighed immediately. The wet weighed brain tissues were transferred to an oven at a constant temperature of 100°C, and the dry weight was obtained after baking. BWC (%) = (wet weight – dry weight)/wet weight × 100%.

### Behavioral test

On the 3<sup>rd</sup> day after operation, the rats were evaluated for their behavioral function through three methods: the modified rat neurological deficit scoring, the forelimb placement test, and the rotation angle test.

#### Evans blue penetration test

EB penetration experiment was performed on the 3<sup>rd</sup> day after cerebral hemorrhage. Rats were injected with 2% w/v EB solution (4 ml/kg) through the femoral vein. After 1 h of body fluid circulation, microscopic observation and spectrophotometric quantification were conducted to detect the permeability of the blood-brain barrier.

#### Immunofluorescence staining

On the 3<sup>rd</sup> day after cerebral hemorrhage, brain tissue was obtained for immunofluorescence staining to evaluate the expression levels of tight junction proteins related to the blood-brain barrier.

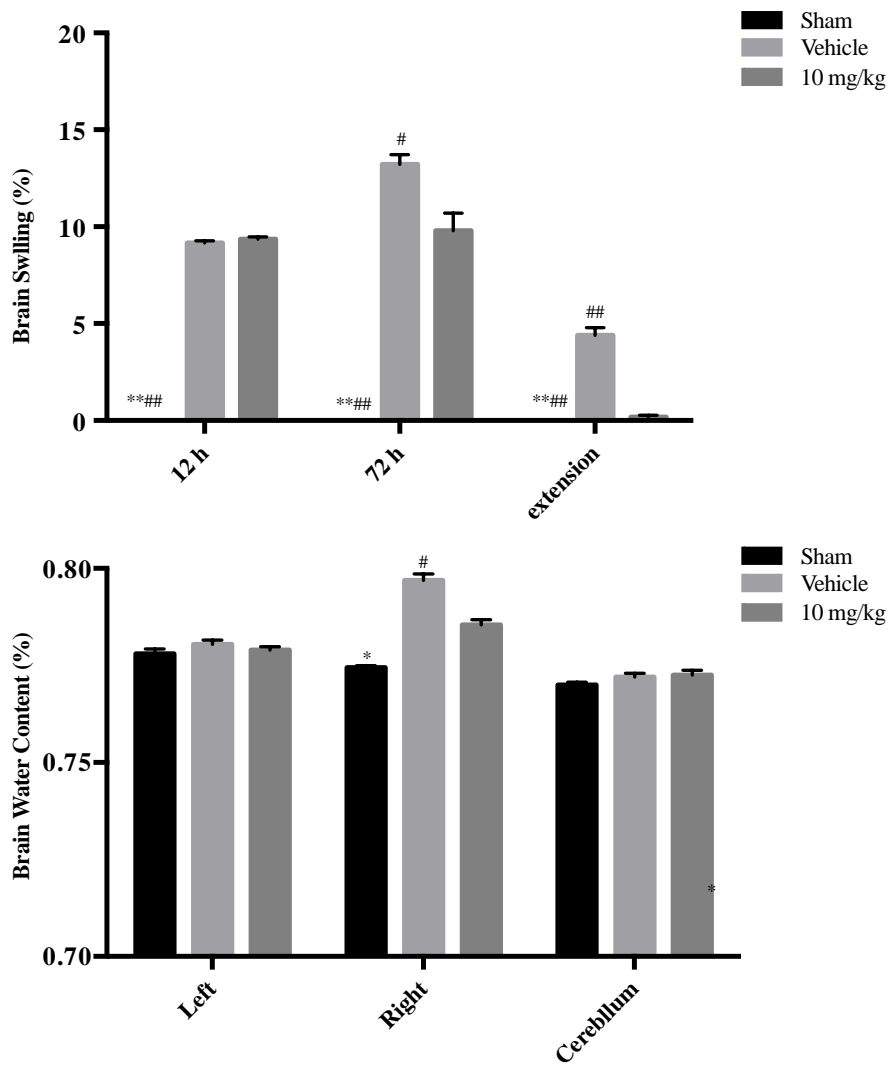
#### Western blotting experiment

On the 3<sup>rd</sup> day after cerebral hemorrhage, brain tissue was obtained for Western blotting to evaluate the expression levels of tight junction proteins related to the blood-brain barrier, from the perspective of the protein level.

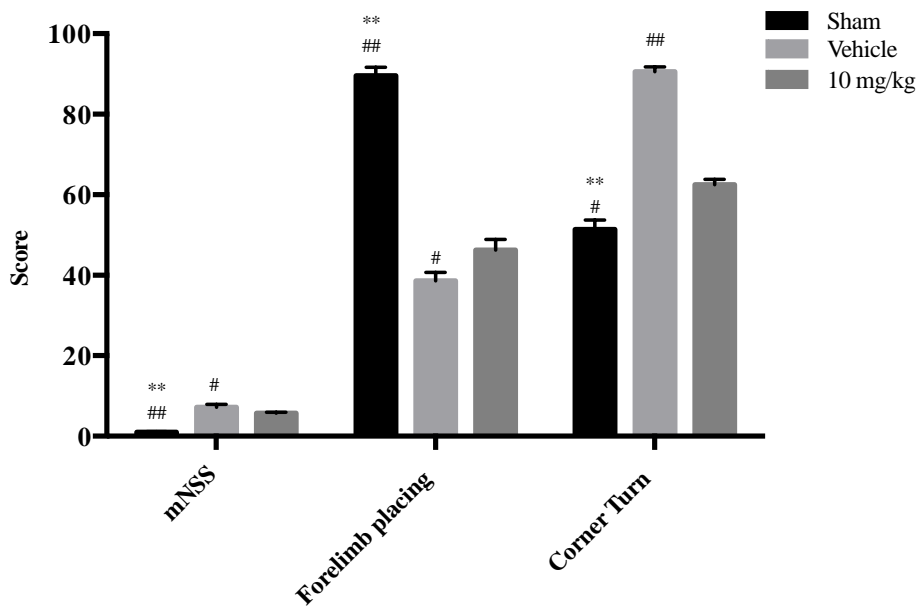
#### Statistical analysis

GraphPad Prism 7.0 software (Dotmatics, Boston, MA, USA) was used for statistical analysis. Data were expressed as mean  $\pm$  standard deviation. The two-tailed *t* test was performed to compare the MRI data of the two groups. Data of brain water content, behavioral function results, EB penetration test, and Western blot results were analyzed by one-way analysis of variance.  $P < 0.05$  of the difference was set as statistically significant. All data analysis was performed blindly by 2 researchers.

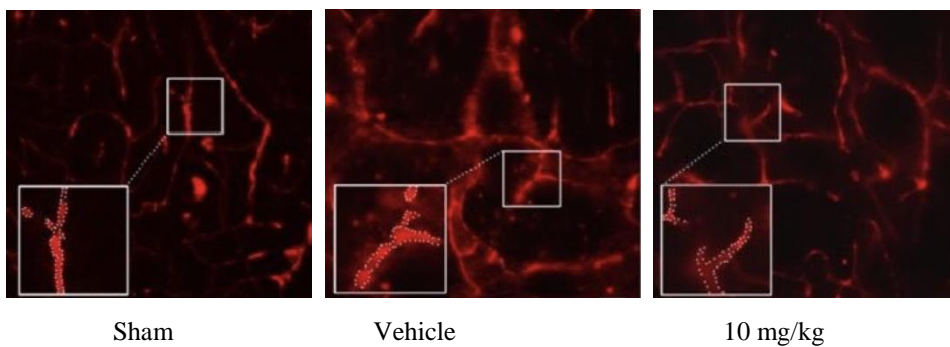
#### Results



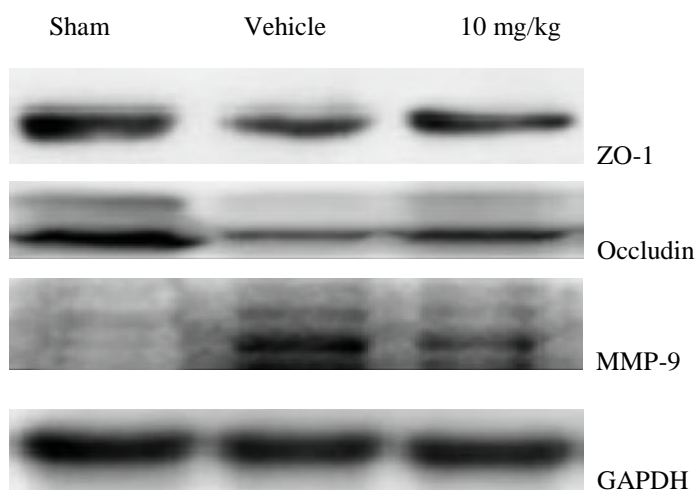
Supplementary Figure S1. The effect of Sanguin on brain edema after cerebral hemorrhage in rats.  $**p < 0.01$  vs. Vehicle;  $^{##}p < 0.01$  vs. 10 mg/kg;  $^{\#}p < 0.05$  vs. 10 mg/kg



Supplementary Figure S2. The influence of Sanguini on the behavior of rats.  $**p < 0.01$  vs. Vehicle,  $*p < 0.05$  vs. Vehicle;  $##p < 0.01$  vs. 10 mg/kg;  $#p < 0.05$  vs. 10 mg/kg



Supplementary Figure S3. The effect of Sanguini on the blood-brain barrier damage caused by cerebral hemorrhage



Supplementary Figure S4. The effect of Sanguini on the expressions of ZO-1, Occludin and MMP-9