Electronic Supplementary Material (ESI) for Food & Function.

Contents

1. Modified neurological severity scores for determining the success of MCAO modeling

After the postoperative anesthetized rats were awake and able to move freely, the success of the model was judged according to the modified neurological severity scoring method (**Tab. 1**, Bieber et al. 2019) and changes in cerebral blood flow, and the neurological function of the rats was scored simultaneously. Rats with neurological deficits were considered as the criteria for successful modeling, and rats with a score of 0 were considered as failed modeling animals and were eliminated. Rats with scores of 1-14 were randomly divided into groups for subsequent experiments.

Table 1. Modified neurological severity scores (Bieber et al. 2019).

Motor tests	
Muscle status-Hemiplegia Raising the rat by the tail:	
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved more than 10° to the vertical axis within 30 s	1
Placing the rat on the floor:	
Inability to walk straight	1
Circling toward the paretic side	1
Fall down to the paretic side	1
Abnormal movements	
Immobility and staring	1
Tremor (wet-dog-shakes)	1
Myodystony, irritability, seizures, myoclonus	1
Sensory tests	
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	1
Reflexes absence of (blunt or sharp stimulation) Pinna reflex (a head shake when touching the auditory meatus)	1
Corneal reflex (an eye blink when lightly touching the cornea with cotton)	1
Startle reflex (a motor response to a brief noise from snapping a clipboard paper)	1
Maximum points	14

One point is awarded for the inability to perform the tasks or for the lack of a tested reflex.
10-14: severe; 5-9: moderate; 1-4: mild injury.

2. Effect of pterostilbene and positive drugs on the body weight of MCAO rats

The rats were weighed every day after MCAO surgery, and the results were shown in **Fig. 1**. On the second day after operation, the weight of the model group was lower than that of drug treatment group. 3 days after operation, the body weight of resveratrol (60 mg/kg) and pterostilbene (60 mg/kg) groups was significantly higher than that of model group, which was 112% and 111% (P<0.05), respectively. The body weight of pterostilbene (15, 30 mg/kg) group was significantly higher than that of model group, which was 116% (P<0.01). At 7 days after operation, the body weight of pterostilbene (60 mg/kg) group was significantly higher than that of model group, which was 119% (P<0.05); The body weight of pterostilbene (15, 30 mg/kg) group was significantly higher than that of model group (127% and 125%, P<0.01). 14 days after surgery, the body weight of pterostilbene (30 mg/kg) group was significantly higher than that of model group (114%, P<0.05), and the body weight of pterostilbene (15 mg/kg) group was significantly higher than that of model group (118%, P<0.01). The research data showed that Pterostilbene could effectively intervene the damage of cerebral ischemia-reperfusion in rats by reducing weight loss and restoring weight gain, and its protective effect is better than that of resveratrol and NBP.

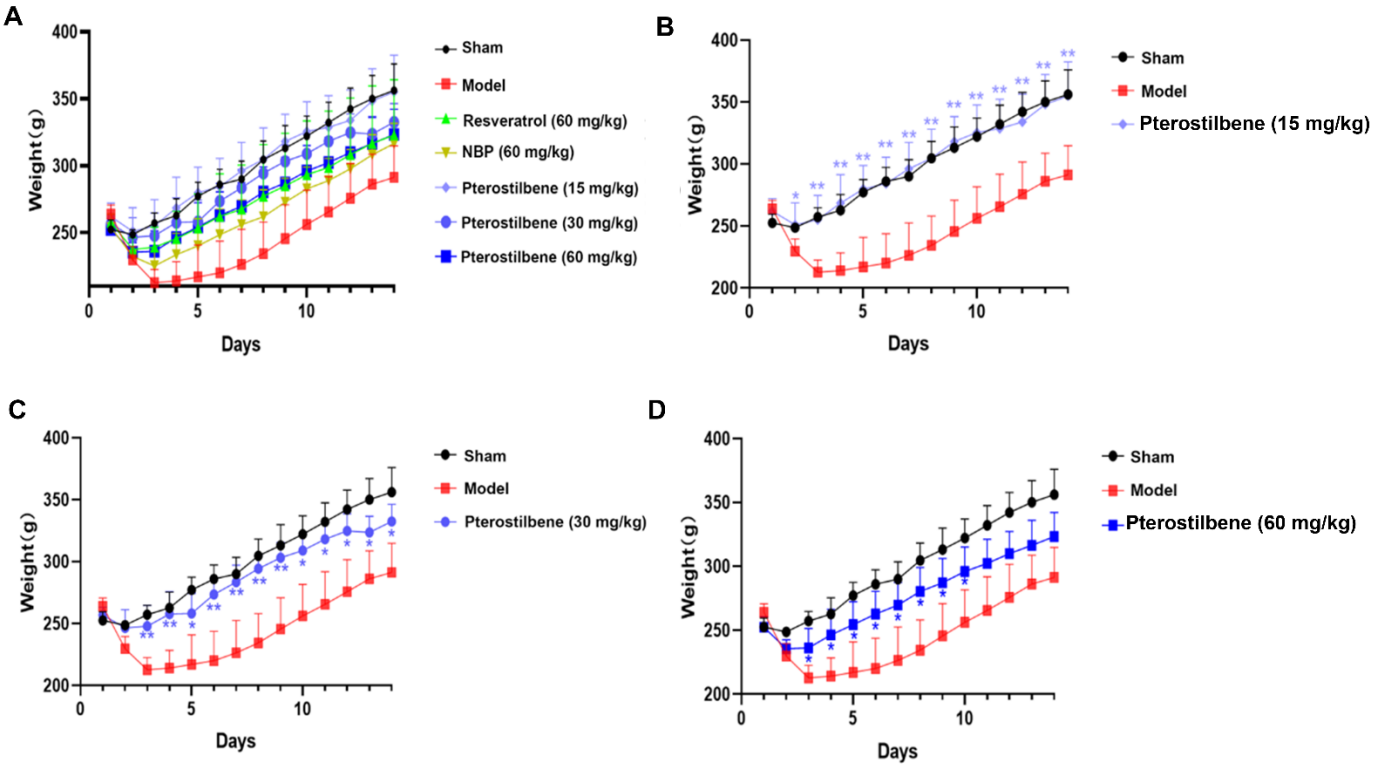

Figure 1. Body weight changes in the rats in each group after oral administration. A Overview of body weight change in each group. **B** Body weight changes in the low-dose pterostilbene group (15 mg/kg). **C** Body weight changes in the medium-dose pterostilbene group (30 mg/kg). **D** Body weight changes in the high-dose pterostilbene group (60 mg/kg). n=6, $^{*}P$<0.05 (vs Model), $^{**}P$<0.01(vs Model).

3. Molecular docking of the ligand resveratrol to receptor 6ESM

The docking score of -6.69 kcal/mol for MOE indicate that resveratrol can interact with receptor MMP-9 directly with a good binding activity.

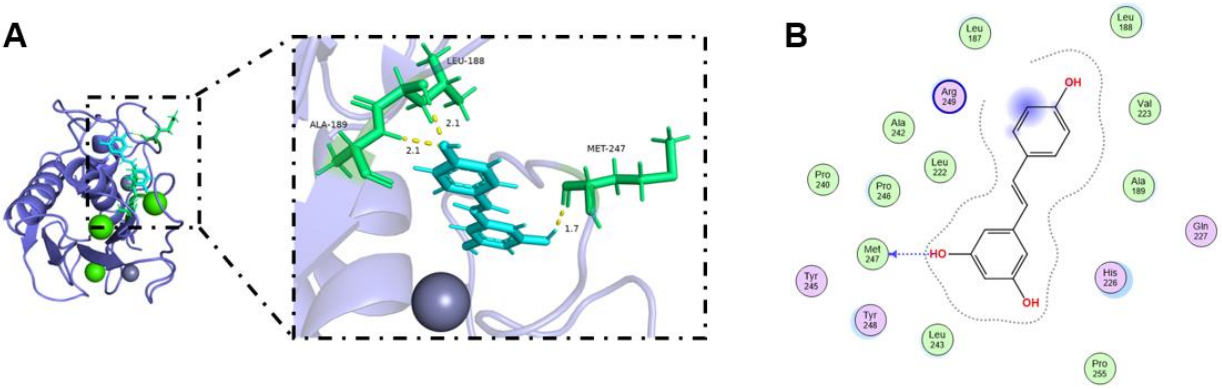

Figure 2. Molecular docking of the ligand Pte to receptor 6ESM. A 3D interaction diagram of MOE docking results. The ligand structure is shown in blue, and the nearby residues are shown in green. The skeleton of the receptor is defined as light purple. **B** 2D interaction diagram of MOE docking results.

4. Exploration and optimization of experimental conditions (parameteres) for glucose and oxygen deprivation

MTT method was used to measure the cell survival rate. After OGD 0.5, 1, 1.5 and 2 h, the experimental results were shown in **Fig. 3**. The relative survival rate of OGD 0.5 h was 91.40 ± 2.30%, and that of OGD 1 h was 85.50 ± 4.46%. The relative survival rate of OGD cells was 32.50 ± 1.66% at 1.5 h and 8.54 ± 0.70% at 2 h. OGD 0.5 h cells had a higher survival rate, but the glucose and oxygen deprivation time was short, and the data were unstable. The relative survival rate of OGD 1.5 h and 2 h cells was low, and the number of cells in subsequent experiments was too small, so 1 h was selected as the duration of subsequent OGD.

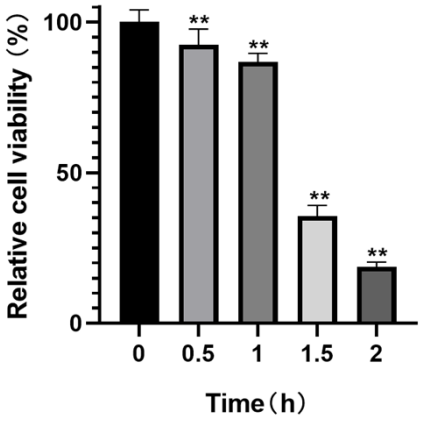

Figure 3. Effect of different duration of glucose and oxygen deprivation on the survival rate of hBMECs. n≥5, $^{**}P$<0.01(vs Control).

5. Laser confocal scanning of the fluorescence wavelength of pterostilbene

A full confocal scan of pterostilbene emission wavelength (STED-inverted confocal microscope, TCS-SP8 STED 3X, Leica) revealed that pterostilbene has an inherent fluorescence with a maximum emission wavelength (λ_{em}) range of 468-484 nm.

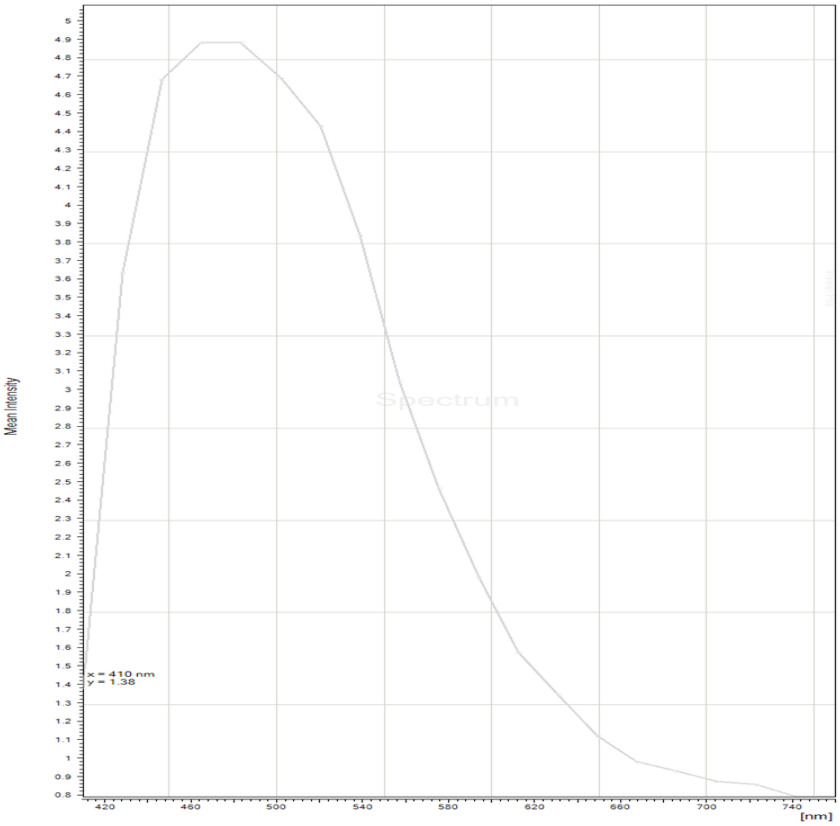

Figure 4. Full confocal scan of pterostilbene emission wavelength.

6. Distribution and localization of pterostilbene in hBMECs

The distribution of pterostilbene in the cell was examined using a confocal laser microscope. It was found that the violet fluorescence of pterostilbene was mainly located on the mitochondria in the cytoplasm, suggesting that the mitochondria may be the main target organelle of pterostilbene. Pterostilbene capable of acting on the nucleus in small amounts for 2 h. It is worth noting that the purple fluorescence of pterostilbene has a very significant distribution and location in cytoskeleton actin.

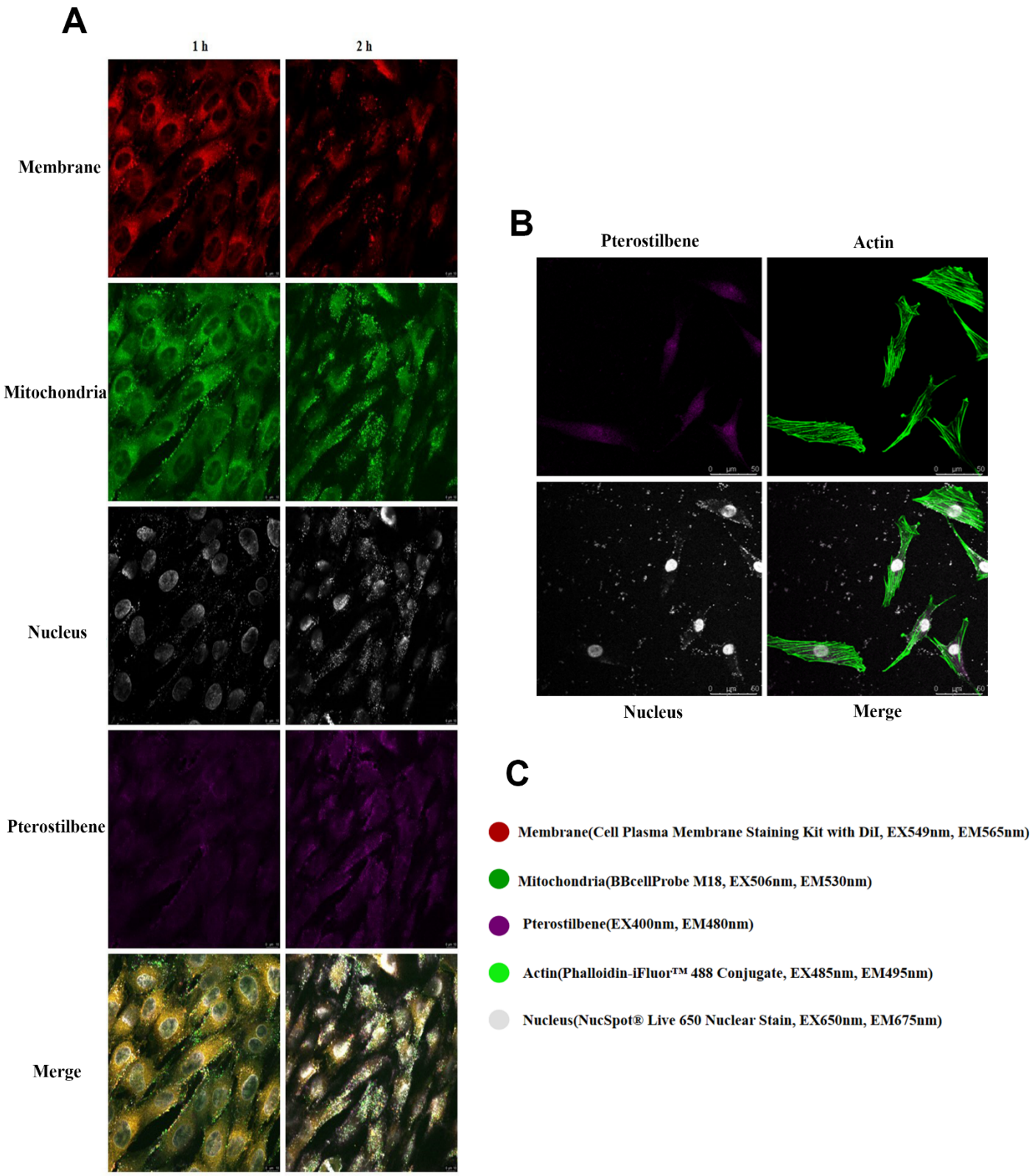

Figure 5. Distribution and localization of pterostilbene in hBMECs. A Biodistribution of pterostilbene in the cytoplasm, mitochondria and nucleus after 1 h and 2 h incubation; scale bar shows 25 μm. **B** Biodistribution of pterostilbene in the actin after 2 h incubation; scale bar shows 50 μm. **C** Fluorescent staining legend of **A**, **B**.

7. Laboratory animal use license

Laboratory Animal Center (license No. SYXK JING 2017-0020) of Institute of medicinal plants, Chinese Academy of Medical Sciences.

8. Ethical approval

Animal Experimentation Ethics Committee of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences: SLXD-20201103012, SLXD-20201117004, SLXD-20201210011, SLXD-20201221041, SLXD-20210409012.

References

Bieber M, Gronewold J, Scharf AC, et al. Validity and Reliability of Neurological Scores in Mice Exposed to Middle Cerebral Artery Occlusion. Stroke. 2019 Oct;50(10):2875-2882.