

Fig. 1. cis-mulberroside A *Ramulus mori*.

2.2. Isolation of cis-mulberroside A

264.7 g of *Ramulus mori* was extracted with 70% ethanol. The extract was concentrated under reduced pressure and extracted with water. The aqueous extract was extracted with ethyl acetate. The ethyl acetate extract was concentrated and reprecipitated with methanol. The methanol-insoluble fraction was dried to give 264.7 g of cis-mulberroside A.

2.3. Animals

Male mice (22–25 g) were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + carrageenan (A+C), and cis-mulberroside A + formalin (A+Fi). The mice were housed in a temperature-controlled environment (22–25 °C) with a 12-h light/dark cycle. The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Carrageenan and formalin were dissolved in distilled water. The mice were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + carrageenan (A+C), and cis-mulberroside A + formalin (A+Fi). The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Carrageenan and formalin were dissolved in distilled water.

2.4. Cell culture

Human umbilical vein endothelial cells (HUVEC) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). The cells were seeded into 96-well plates at a density of  $1 \times 10^6$  cells per well. The cells were cultured for 24 h in the presence of cis-mulberroside A (1  $\mu$ g/ml) or formalin (100  $\mu$ g/ml). The cell viability was determined by measuring the optical density (OD) at 550 nm. The OD values were normalized to the control group (C). The results are expressed as the mean  $\pm$  SD of three independent experiments. Statistical significance was determined by one-way ANOVA. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

1  $\mu$ g/ml cis-mulberroside A (1  $\mu$ g/ml) was added to the culture medium. The cells were cultured for 24 h. The cell viability was determined by measuring the optical density (OD) at 550 nm. The OD values were normalized to the control group (C). The results are expressed as the mean  $\pm$  SD of three independent experiments. Statistical significance was determined by one-way ANOVA. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

2.5. Acetic acid-induced abdominal constrictions and peritoneal capillary permeability in mice

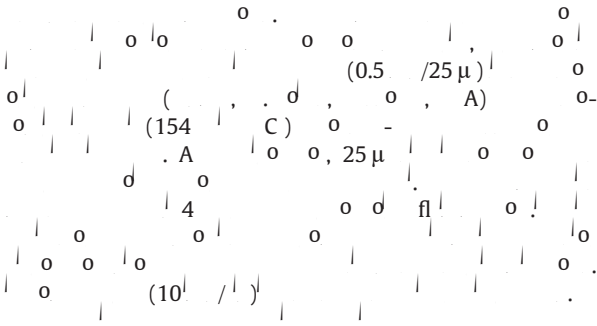
Male mice (22–25 g) were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + acetic acid (A+A), and cis-mulberroside A + formalin (A+Fi). The mice were housed in a temperature-controlled environment (22–25 °C) with a 12-h light/dark cycle. The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Acetic acid and formalin were dissolved in distilled water. The mice were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + acetic acid (A+A), and cis-mulberroside A + formalin (A+Fi). The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Acetic acid and formalin were dissolved in distilled water.

2.6. Formalin test

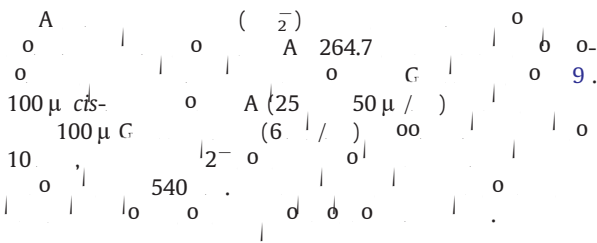
Male mice (22–25 g) were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + formalin (A+Fi), and cis-mulberroside A + carrageenan (A+C). The mice were housed in a temperature-controlled environment (22–25 °C) with a 12-h light/dark cycle. The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Formalin and carrageenan were dissolved in distilled water. The mice were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + formalin (A+Fi), and cis-mulberroside A + carrageenan (A+C). The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Formalin and carrageenan were dissolved in distilled water.

2.7. Carrageenan-induced hind paw edema model

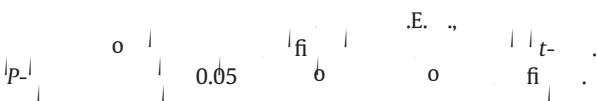
Male mice (22–25 g) were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + carrageenan (A+C), and cis-mulberroside A + formalin (A+Fi). The mice were housed in a temperature-controlled environment (22–25 °C) with a 12-h light/dark cycle. The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Carrageenan and formalin were dissolved in distilled water. The mice were divided into four groups: control (C), cis-mulberroside A (A), cis-mulberroside A + carrageenan (A+C), and cis-mulberroside A + formalin (A+Fi). The mice were fasted overnight (12 h) before the experiment. The cis-mulberroside A was dissolved in distilled water. Carrageenan and formalin were dissolved in distilled water.



2.8. Nitrite analysis



2.9. Statistical analysis



3. Results

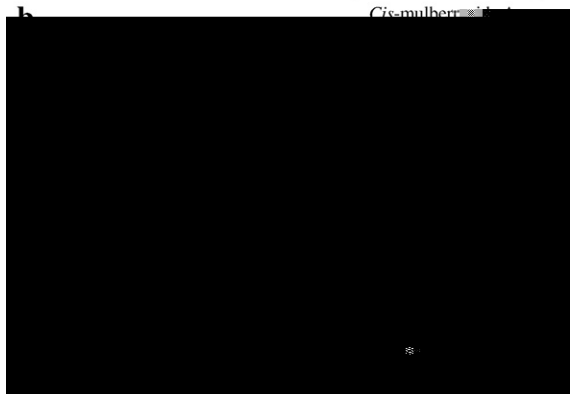
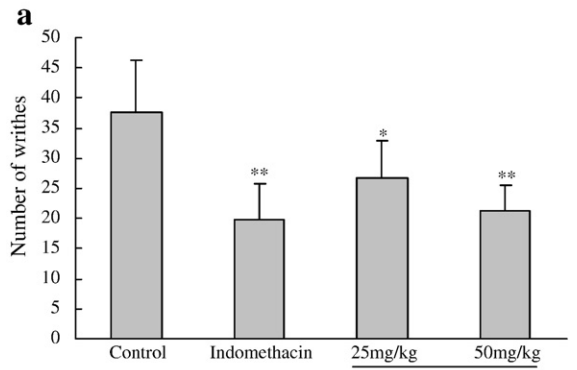
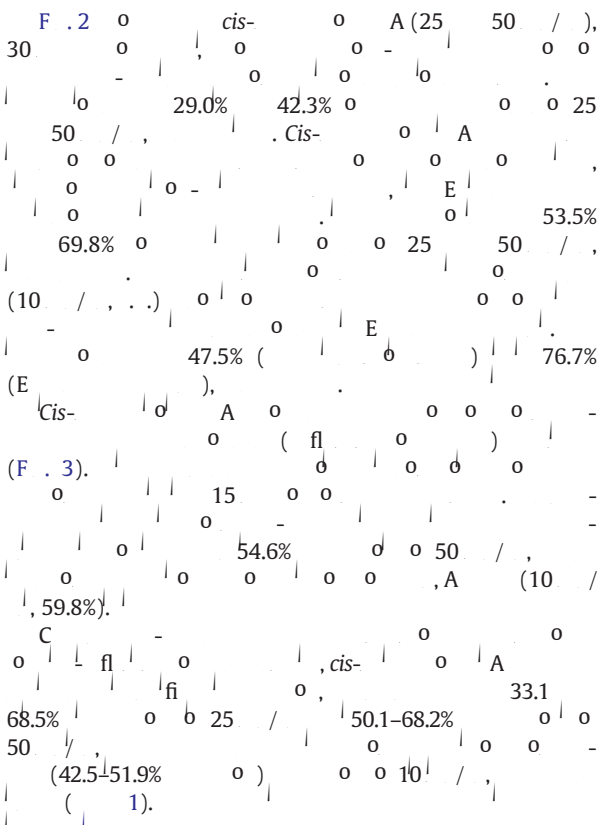


Fig. 2. Effect of cis-mulberoside A on the number of writhes in response to 100 μg/ml of indomethacin. The number of writhes was significantly reduced by cis-mulberoside A at 25 mg/kg and 50 mg/kg. \*P < 0.05, \*\*P < 0.01. N = 8.

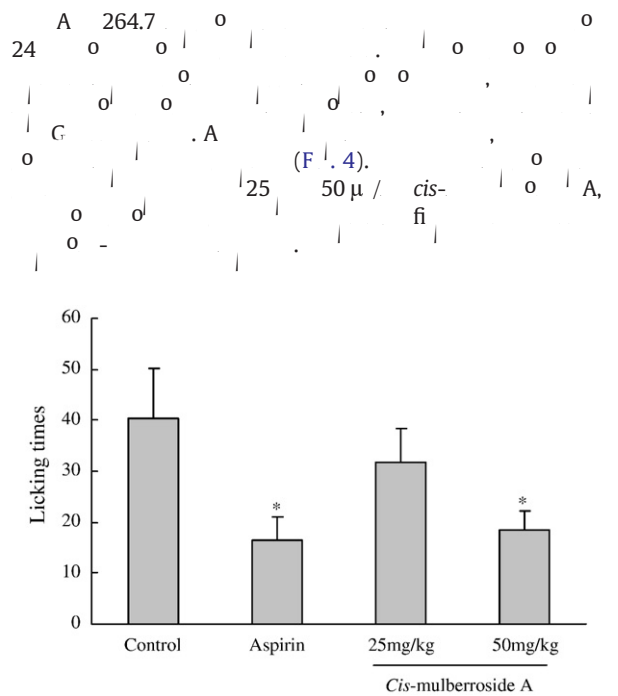
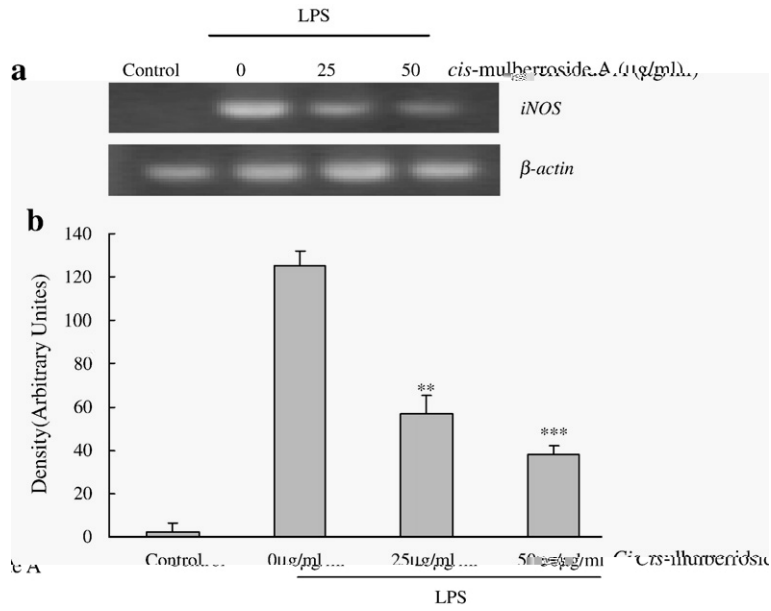


Fig. 3. Effect of cis-mulberoside A on the licking times in response to 150 μg/ml of aspirin. The licking times were significantly reduced by cis-mulberoside A at 25 mg/kg and 50 mg/kg. \*P < 0.05. N = 8.





**Fig. 5.** Effect of cis-mulheroside on LPS-induced iNOS expression in *R. mori*. *R. mori* cells were treated with LPS (1.0 µg/ml) and cis-mulheroside (0, 25, 50 µg/ml) for 4 h. The cell lysates were analyzed by Western blotting with anti-iNOS and anti-β-actin antibodies. The bar graph shows the relative density of iNOS protein. Values are expressed as mean ± SD. \*\*P < 0.01; \*\*\*P < 0.001.

18–20  
21

*R. mori*

A 264.7

cis-

A 264.7

*R. mori*

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (No. 30571406), the National Science and Technology Major Project (No. 2003 F005).

**References**

1. ... 2005;31:348–50.
2. ... 2002;20:467–9.
3. ... 2008;26:325–30.
4. ... 2002;22:1369–78.
5. ... 2005;48:1021–34.
6. ... 1985;14:69–76.
7. ... 2005;98:201–6.
8. ... 2007;110:504–15.
9. ... 1993;191:1301–8.
10. ... 1997. 1–20.
11. ... 1996;75:2361–8.
12. ... 1987;30:103–14.
13. ... 1997;31:381–9.
14. ... 2002;924:219–28.
15. ...
16. ... 2003;75:115–21.
17. ... 2007;114:355–63.
18. ... 2006;104:410–4.
19. ... 1994;305:253–64.
20. ... 1995;3:2–32.
21. ... 1997;15:323–50. 2001;67:103.