Supplementary Document

Mori Cortex regulates P-glycoprotein in Caco-2 cells and colons from rats with experimental colitis via direct and gut microbiota-mediated mechanisms

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Materials and Methods

In vitro cytotoxicity and anti-proliferation studies

Caco-2 cells were cultured in DMEM supplemented with 10% Fetal Bovine Serum (FBS), 1% of a mixture of penicillin and streptomycin (P/S) and 1% nonessential amino acids (NEAA), in an atmosphere containing 5% CO_2 and 90% relative humidity at 37 ℃. Cells were sub-cultured every 3-4 days after reaching 80-90% confluence by trypsinization with 0.05% trypsin-EDTA.

The cytotoxicity of MCE and the main components (mulberroside A (MA), oxyresveratrol (Oxy), resveratrol (Res), morusin (Ms), sanggenon C (SC), kuwanon G (KG), Morin) on Caco-2 cells was measured by MTT assay. Caco-2 cells were seeded onto a 96-well plate at a density of 1×10^4 cells/well in DMEM culture medium. After 24 h culturing at 37 ℃, the medium was replaced with different concentrations of MCE (0-250 μg/mL) or 0-50 μM of components (MA, Oxy, Res, Ms, SC, KG, Morin) dissolved in Hank's balanced salt solution (HBSS)-4-(2-hydroxyethyl)-1- piperazineethane sulfonic acid (HEPES) (10 mM of HEPES, pH 7.4). Then cells were incubated at 37 ℃ in the CO_2 incubator for 24 h. The solution in each well was aspirated, and the cells were incubated for a further 4 h with 200 μl of MTT solution (1 mg/ml in HBSS-HEPES). The intracellular formazan product was solubilized with 200μl of DMSO, and absorbance was measured at 570 nm using Spectra Max M5 Multi-Mode Microplate Readers (Molecular Devices, USA). And the viability of MCE-exposed cells was expressed as the percentage of the absorbance measured in the

control cells.

For the anti-proliferation study, the cells were seeded on 96-well plates for 24 h followed by incubating with 0-250 μg/mL of MCE, and 0-50 μM of components (MA, Oxy, Res, Ms, SC, KG, Morin) in culture medium for another 72 h. The cells were then washed thrice with HBSS buffer after medium aspiration, and examined by MTT assay as described above.

Supplementary Figures

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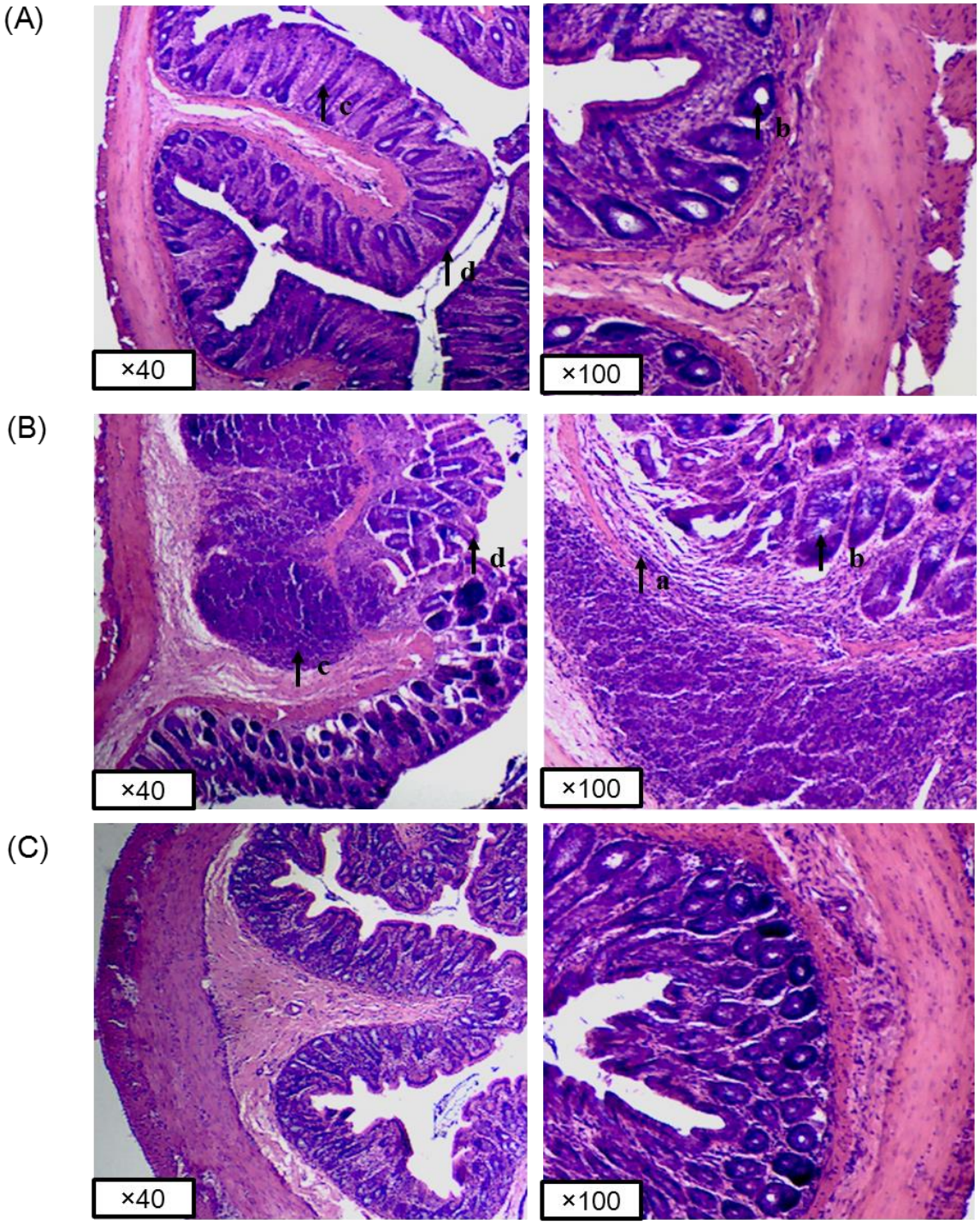
Supplementary Figure legends

Supplementary Fig. S1. Histological assessment of the colon tissues in normal rats (A), rats with colitis (B) and those receiving MCE treatment (C). Representative photomicrographs (H&E staining, original magnification ×40 & ×100) of colonic tissues showed intact epithelial surface in normal rats and severe destruction of epithelium with inflammatory cell infiltration (a), crypt abscess (b), loss of goblet cells (c) and epithelium (d) in rats with DSS-induced colitis. Treatment with MCE attenuated the extent and severity of cell damage.

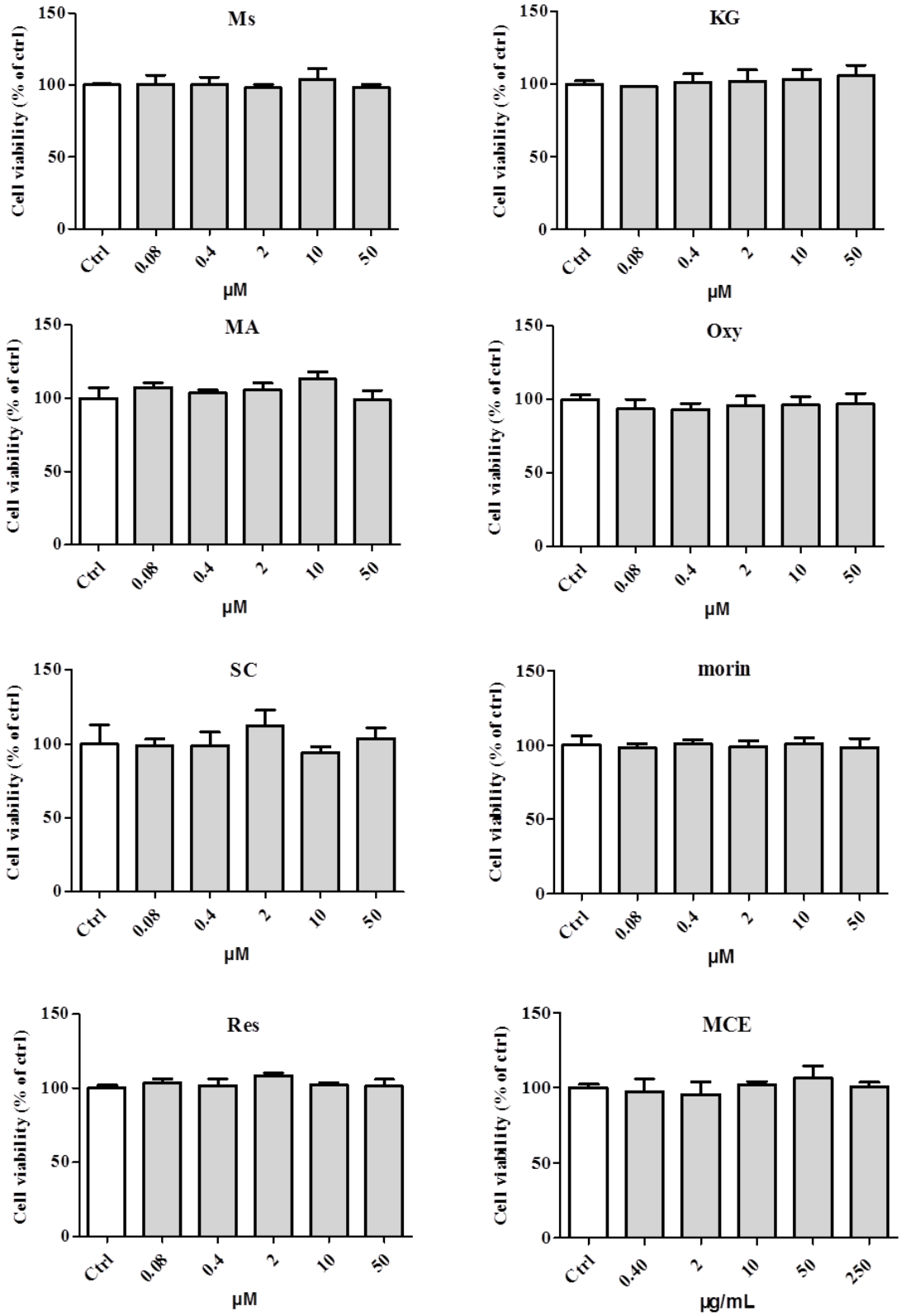
Supplementary Fig. S2. Cytotoxicity effects of MCE and its major components on Caco-2 cells. Caco-2 cells was exposed to series concentrations of Ms(morusin), KG(kuwanon G), MA (mulberroside A), Oxy(oxyresveratrol), SC (sanggenon C), morin, Res(resveratrol) and MCE (Mori Cortex extract) for 24 h. Cell viability was detected with MTT assay and presented as percent of control. Data were expressed as mean ± SD from triplicate experiments.

Supplementary Fig. S3. Short-term (a) and long-term (b) effects of main components of MCE on P-gp expression in Caco-2 cells. Cells were treated with 50 μM of kuwanon G (KG), morusin (Ms), morin, oxyresveratrol (Oxy), resveratrol (Res), mulberroside A (MA) and sanggenon C (SC) for 24 h or 50 μM of MS, morin, MA, SC and KG for 7 days,

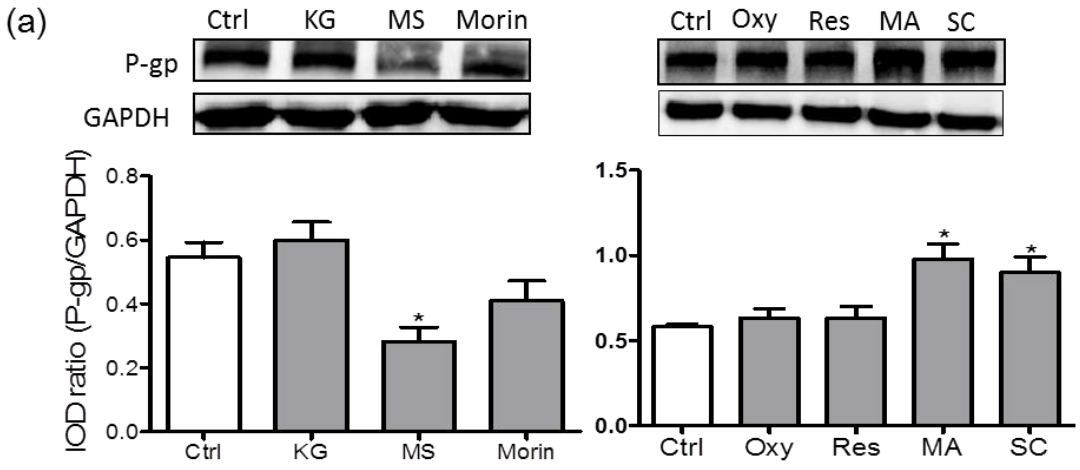
respectively. The P-gp expression was evaluated using Western blot. Data represented mean ± SD from triplicate experiments. *$p < 0.05$ *vs.* control (Ctrl) group (unpaired student's *t* test).

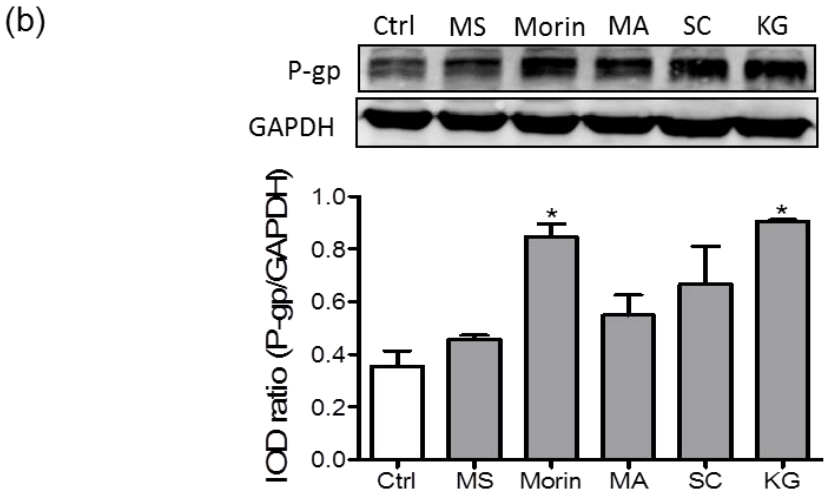


Supplementary Fig. S1



Supplementary Fig.S2





Supplementary Fig.S3