Supporting Information

Characterization of in Vitro ADME Properties of Diosgenin and Dioscin from Dioscorea villosa

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Methods and Results:

Determination of diosgenin and dioscin cytotoxicity in Caco-2 cells:

To determine if diosgenin and dioscin were cytotoxic to Caco-2 cells, CellTiter 96® AQueous one solution cell proliferation assay (Promega Corporation) was used according to the manufacturers guidelines. Briefly, 20,000 cells per well in 100 µL DMEM media were seeded in 96-well plates. After 24h, diosgenin (200 µM), dioscin (100 µM), and positive control Triton X-100 (0.25%) were added. After 2h incubation at 37 ºC, 20 µL of MTS [3-(4,5- methylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt] dye was added and incubated for 1h at 37 ºC. After 1h, the absorbance of the plate was read at 490 nm on a Spectramax M5 plate reader. Percent cell viability was calculated compared to the control. No cytotoxicity was observed with either diosgenin (200 µM) or dioscin (100 µM) in Caco-2 cells, while Triton X-100 (0.25%) showed significant cytotoxicity (Figure 1S). These results suggest that the concentrations of diosgenin and dioscin used in bi-directional transport experiment had no effect on the viability of Caco-2 cells.

Figure 1S: Cell viability of Caco-2 cells in response to diosgenin and dioscin (2 h treatment) as determined by MTS assay. Triton X-100 (0.25%) was used as positive control. *** p<0.05, determined by Kruskal-Wallis test. The data shown are mean ± SD of triplicate treatments in one experiment.
Figure 1S

![Graph showing cell viability](image)

- **DMSO (0.5%)**
- **Diosgenin (200μM)**
- **Dioscin (100μM)**
- **Triton X-100 (0.25%)**