

Supplementary Materials

In this experiment, we explored the synergistic effects of emodin (EMO) and glycyrrhizin (GL) against renal fibrosis and screened for optimal molar ratio (Fig. S1-S2). The synergistic effect of two drugs was verified using MTT assay in NIH3T3 cells. Both Gaddum model and Chou-Talalay method were applied to analyze the synergism. Results showed that combined use of EMO and DAG could exert synergistic effect when the mole ratio between EMO and DAG altered from 1:2 to 1:10. In addition, in order to further investigate the anti-renal fibrosis effect of the two drugs through inhibiting the proliferation and transdifferentiation of fibroblasts, myofibroblast model is established through TGF- β 1 induction (Fig.S3). According to the results of cell proliferation inhibition experiments, myofibroblast model was treated with EMO, GL and EMO/GL (molar ratio at 1:5) for 48h, respectively, followed by Western blot analysis of α -smooth muscle actin (α -SMA), transforming growth factor- β 1 (TGF- β 1), collagen type 1 (Col1) and fibronectin (FN). Results showed that renal fibrosis-associated protein expression including α -SMA, TGF- β 1, Col-I and FN were significantly decreased after treated with combination of EMO and DAG (Fig.S4).

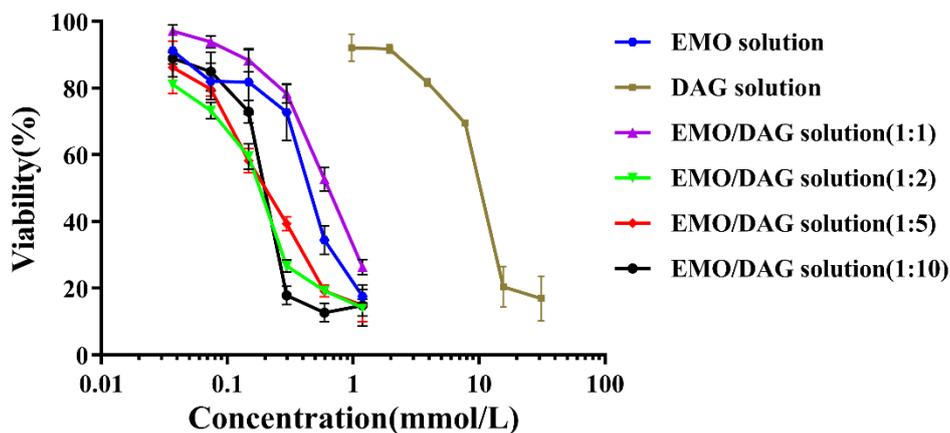


Figure S1. Cell viability of NIH3T3 cells after treatment with various formulations.

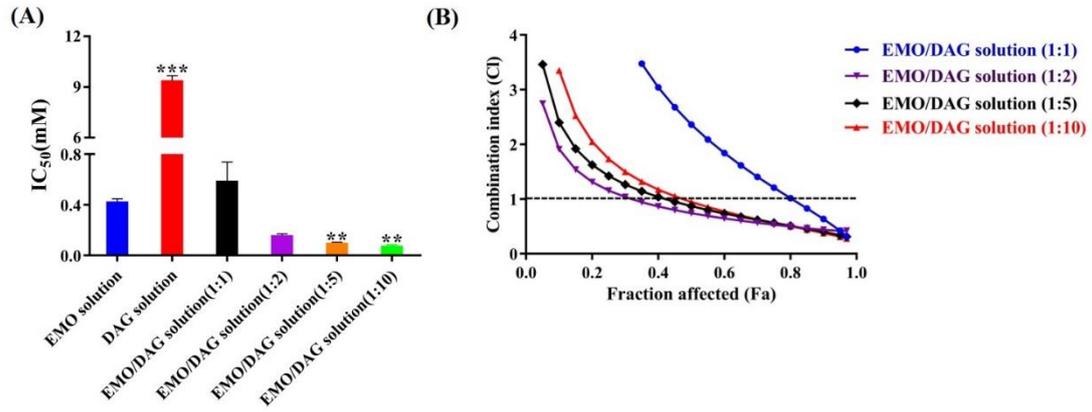


Figure S2. The IC₅₀ values (A) and combination index (CI) (B) in NIH3T3 cells of various formulations. (*P<0.05, **P<0.01, ***P<0.001 compared to EMO solution group.)

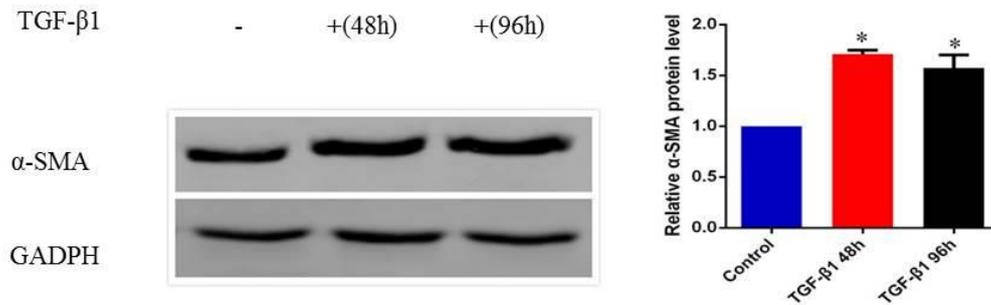
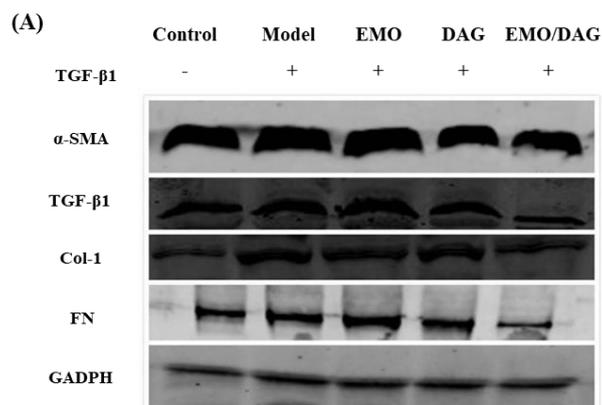


Figure S3. The expression of α-SMA protein in NIH3T3 cells induced by TGF-β1 (5ng/mL). (*P<0.05, compared to control group).



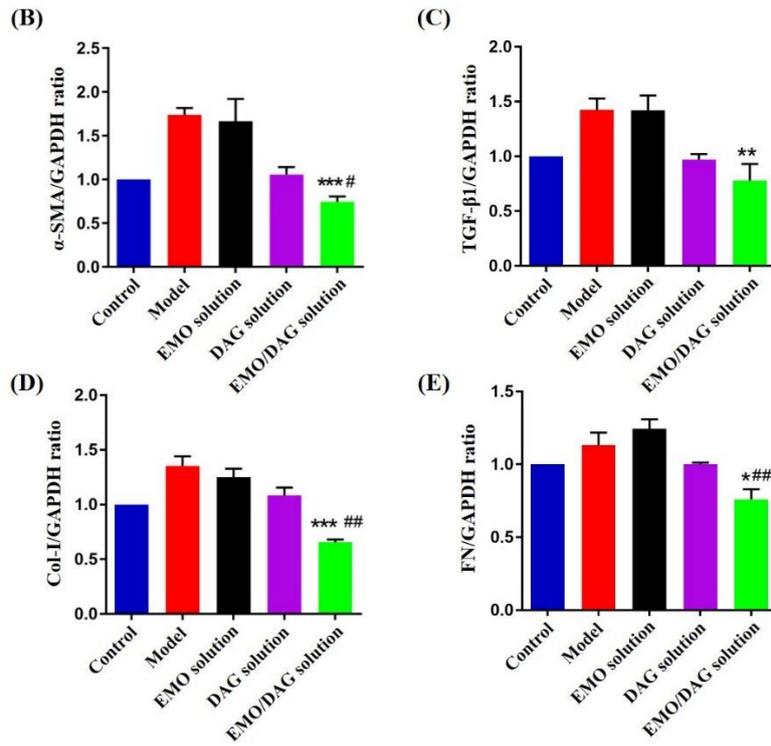


Figure S4. Effects of various formulations on protein levels in NIH3T3 cells. A comprehensive images of renal fibrosis-related proteins (α -SMA, TGF- β 1, Col-I and FN). B-E represents the relative protein quantification of α -SMA, TGF- β 1, Col-I and FN in NIH3T3 cells, respectively. (* P <0.05, ** P <0.01, and *** P <0.001, compared to control group. # P <0.05 and ## P <0.01, compared to EMO solution group.)