Supplementary information

Orally administrable polyphenol-based nanoparticle achieves anti-inflammation and antitumor treatment of colon diseases

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1. Supplementary methods

1.1. In vitro cytotoxicity assay

The cytotoxic activity of tannic acid, TPGS, and TTNP against HT-29 cells or RAW 264.7 cells was tested by the standard MTT assay. Briefly, HT-29 cells or RAW 264.7 cells at an initial density of 1×10^4 cells/well were seeded into 96-well plates and incubated for 24 h, respectively. Then, the culture media were replaced with fresh media containing various samples at an equal tannic aicd dose of 10 μg/mL, respectively. After incubation of 24 h, relative cell viability was tested by our previously described methods.[1]

To evaluate in vitro anticancer effect of DTX-TTNP. HT-29 cells at an initial density of 1×10^4 cells/well were seeded into 96-well plates and incubated for 24 h. Then culture media were replaced with fresh media containing free DTX or DTX-TTNP at equal DTX doses of 0.01, 0.04, 0.2, 1, 5, or 25 μg/mL, respectively. After separate incubation for another 24 h or 48 h, cell cytotoxicity was tested by the same procedures as described above.

1.2. In situ single-pass intestinal perfusion (SPIP)

In situ SPIP experiment was performed as previously described with some modifications.[2] Briefly, male Sprague-Dawley (SD) rats (from the Animal Experimental Centre, Sun Yat-sen University) were anesthetized and placed in prewarmed plates, then the abdomen opened along the midline, and different intestinal segments including duodenum, jejunum, ileum or colon were carefully exposed in 10 cm length. After washing with prewarmed saline, intestinal segments were equilibrated with Krebs Ringer's buffer containing DTX or DTX-TTNP (10 μg/mL of DTX) for 45 min and then continue with the perfusion process for 60 min. The flow rate of perfusate was set at 0.2 mL/min, and donor and receptor vials were replaced and weighted per 15 min. Afterward, the length and radius of each tested intestinal segment were recorded, and absorption rate (K_a) and apparent permeability (P_{app}) were calculated as the following equations 1 and 2:

$$K_a = \left(1 - \frac{C_{out}}{C_{in}} \cdot \frac{V_{out}}{V_{in}}\right) \cdot \frac{Q}{\pi r^2 l} \quad (1)$$

$$P_{app} = \frac{-Q \cdot \ln\left(\frac{C_{out}}{C_{in}} \cdot \frac{V_{out}}{V_{in}}\right)}{2\pi r l} \quad (2)$$

where C_{out} and C_{in} are DTX concentration in the receptor and donor vial, respectively, V_{out} and V_{in} are the volume of perfusate in the receptor and donor vial, respectively, Q is the flow rate of perfusate, r and l are the radius and length of the tested intestinal segment, respectively.

2. Supplementary figures

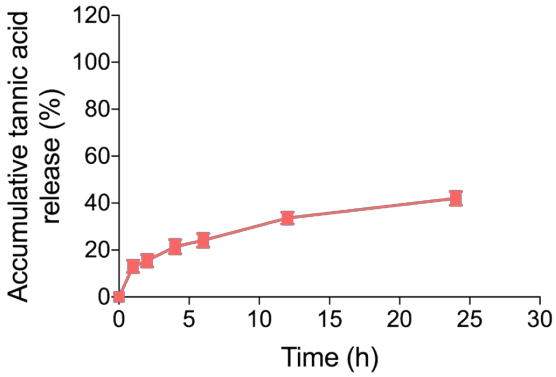

Fig. S1 In vitro accumulative release profiles of tannic acid from DTX-TTNP in pH 7.4.

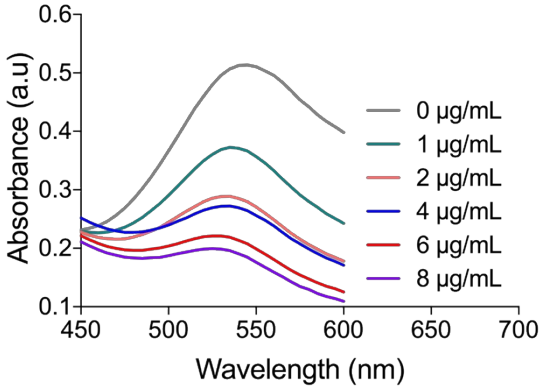

Fig. S2 UV-visible spectra of DPPH radicals after treatment with TTNP at various tannic acid concentrations.

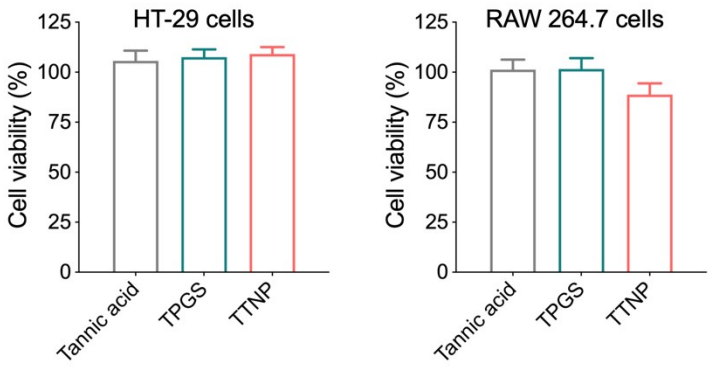

Fig. S3 In vitro cytotoxicity of HT-29 cells and RAW 264.7 cells after co-incubation with tannic acid, TPGS, or TTNP for 24 h, respectively. Tannic acid was 10 μg/mL (n = 3).

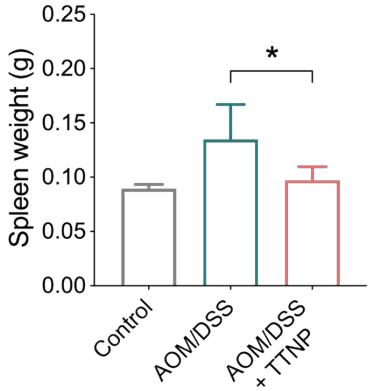

Fig. S4 Spleen weight of mice at the end of prevention therapy study (day 49). *$P < 0.05$ (n = 5).

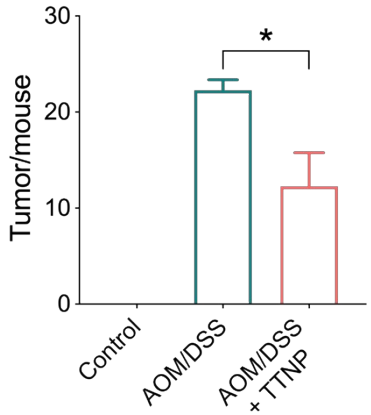

Fig. S5 Colorectal tumor numbers of mice at the end of prevention study (day 49). *$P < 0.05$ (n = 5).

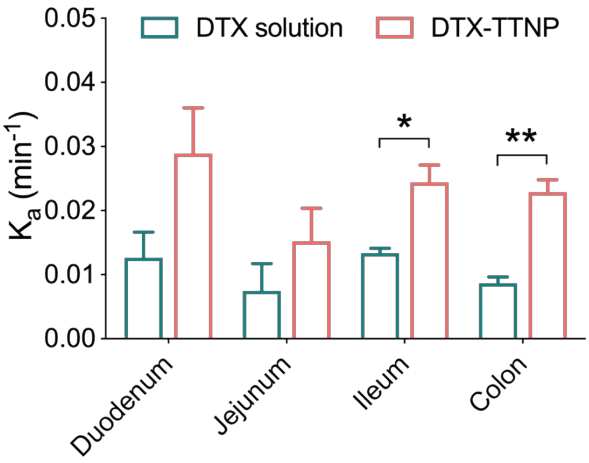

Fig. S6 K_a values of duodenum, jejunum, ileum and colon, as measured by in situ single-pass intestinal perfusion experiment. *$P < 0.05$, **$P < 0.01$ (n = 3).

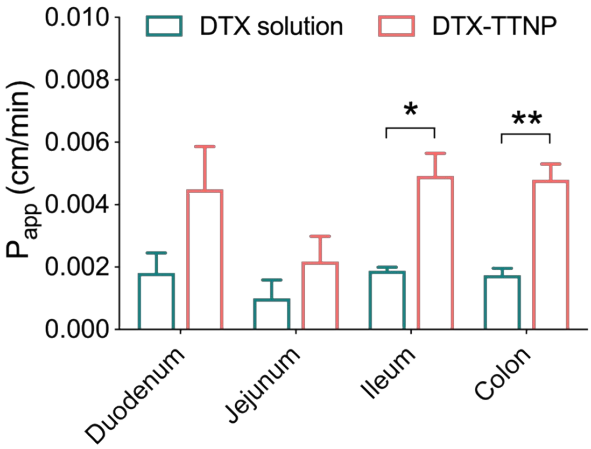

Fig. S7 P_{app} values of duodenum, jejunum, ileum and colon, as measured by in situ single-pass intestinal perfusion experiment. *$P < 0.05$, **$P < 0.01$(n = 3).

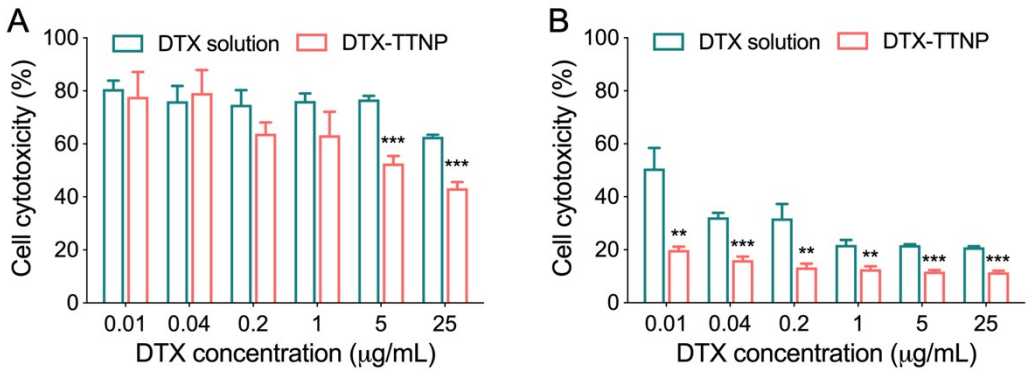

Fig. S8 (A, B) In vitro cytotoxicity after HT-29 cells treated with various concentrations of DTX or DTX-TTNP for 24 h (A) or 48 h (B), respectively, **p<0.01, ***p<0.001 (n = 3).

References

1. Z. Le, Z. Liu, L. Sun, L. Liu and Y. Chen, *ACS Appl. Bio Mater.*, 2020, **3**, 5202–5212.

2. D. Zhang, X. Pan, S. Wang, Y. Zhai, J. Guan, Q. Fu, X. Hao, W. Qi, Y. Wang, H. Lian, X. Liu, Y. Wang, Y. Sun, Z. He and J. Sun, *Mol. Pharm.*, 2015, **12**, 2337–2351.