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Supporting Information

Aminocellulose-grafted polycaprolactone coated gelatin nanoparticles mediated dual drug delivery ameliorates severity of ulcerative colitis: A novel adjuvant therapeutic approach

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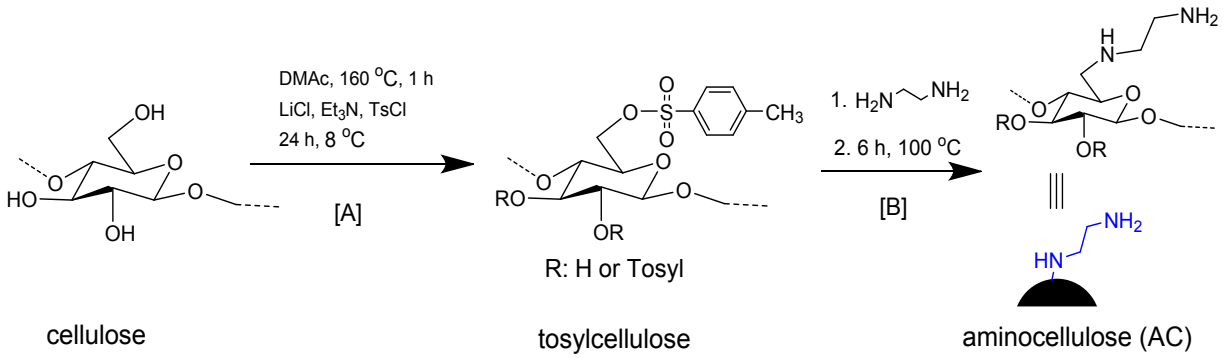
1. Formulation and characterization of PCL-AC-coated gelatin nanoparticles

1.1. Synthesis of aminocellulose



Scheme SC1. Synthesis of aminocellulose from cellulose and tosylcellulose (with permission from[1] copyright (2018) American chemical Society).

1.2. Grafting of aminocellulose (AC) over polycaprolactone (PCL)

Aminocellulose grafted polycaprolactone (PCL-AC) was synthesized by amidation reaction as described previously.[2] Polycaprolactone (PCL) was dissolved in dichloromethane (DCM) and aminocellulose (AC) was dissolved in methanol (PCL : AC :: 1:1 equivalents). The two solutions were placed in separate addition funnels and added dropwise into a round bottom flask and were reacted for 48 hours at 60°C. After the stipulated time, the reaction was stopped, solvent was evaporated using rotavapor (Rotavapor® R-300) and final precipitate obtained was washed twice with methanolic aqueous solution with gentle stirring to remove the unreacted aminocellulose. The product was vacuum dried overnight at 35°C and characterized by various techniques.

1.3. Characterization of aminocellulose-grafted polycaprolactone (PCL-AC)

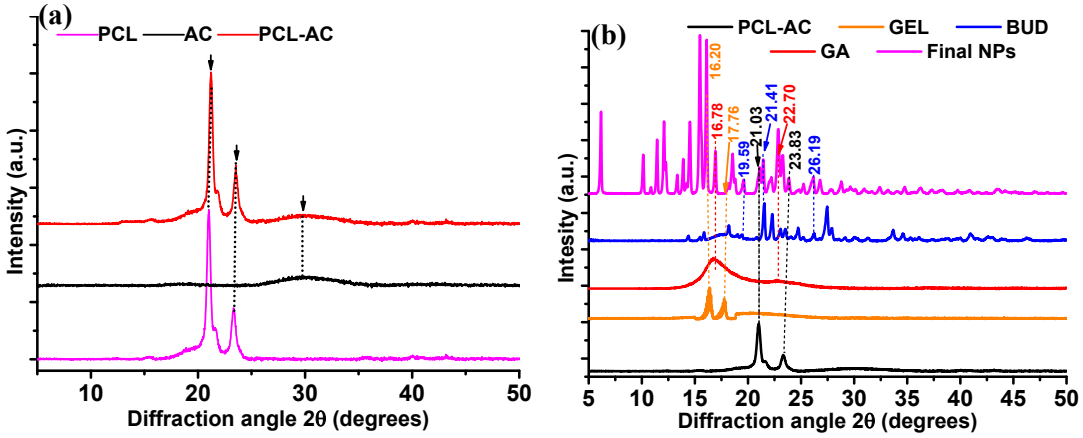


Fig. S1. (a) Comparative XRD spectra of polycaprolactone (PCL) (pink), aminocellulose (AC) (black) and aminocellulose-grafted polycaprolactone (PCL-AC) (red) (b) Comparative XRD spectra of PCL-AC (black), gelatin (orange), glycyrrhizic acid (red), budesonide (blue) and final DDL nanoparticles (pink) (with permission from[2] Copyright © 2021 Elsevier Ltd.).

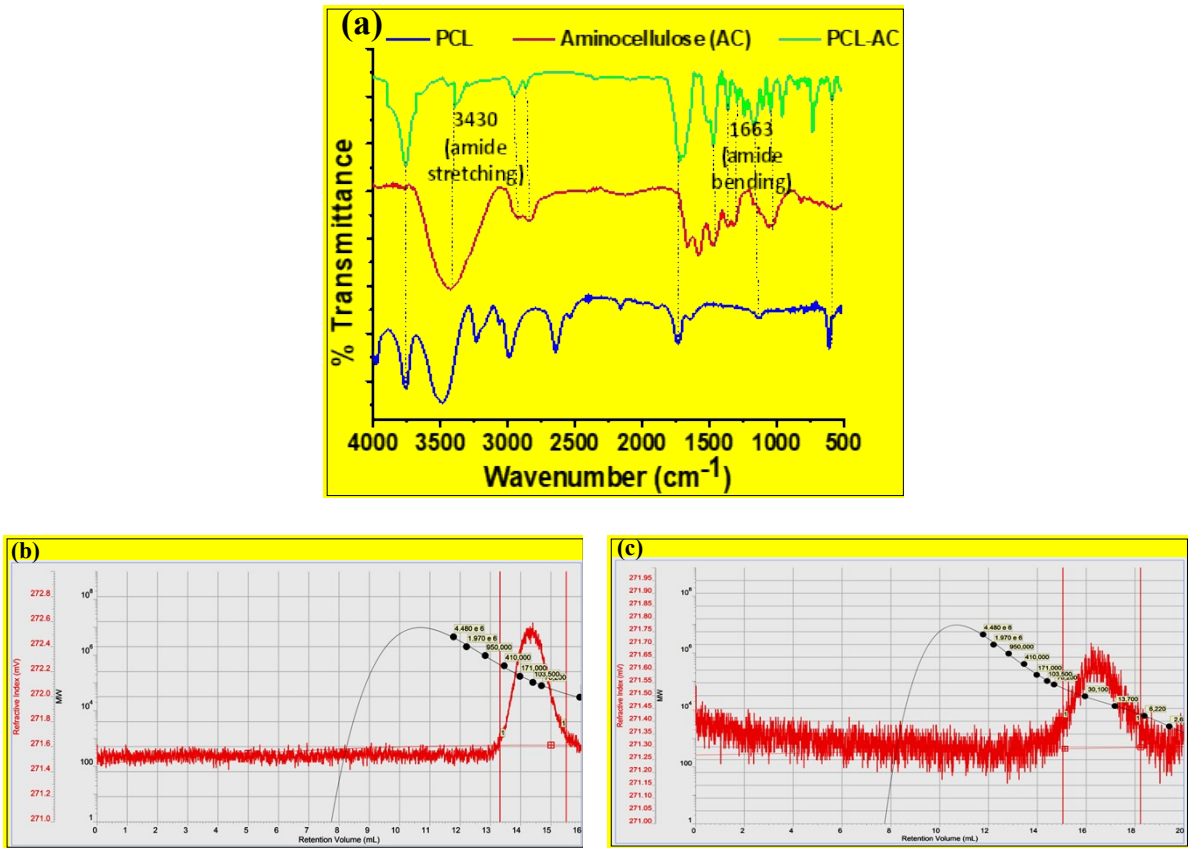


Fig. S2. (a) Comparative Fourier Transform Infra-red (FT-IR) spectroscopic analysis of polycaprolactone (PCL) (blue), aminocellulose (AC) (red) aminocellulose grafted polycaprolactone (PCL-AC) (green). Dotted lines indicate peaks common to PCL, AC and PCL-AC graphs. **(b)** Gel permeation chromatography curves of polycaprolactone (PCL) and **(c)** aminocellulose grafted polycaprolactone (PCL-AC) (with permission from[2] Copyright © 2021 Elsevier Ltd.)

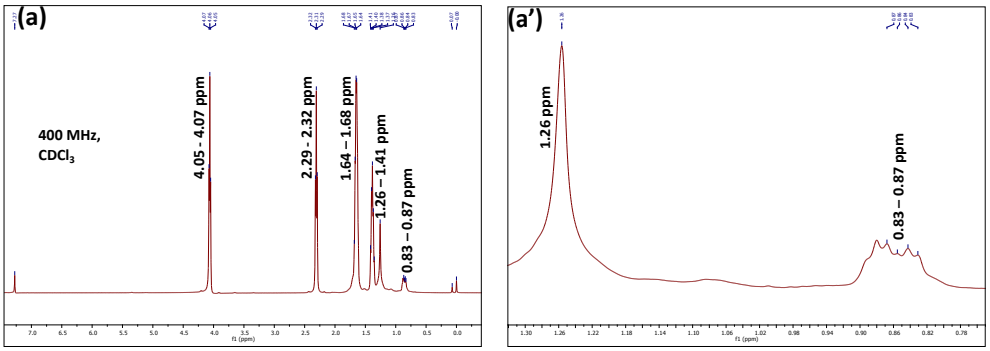


Fig. S3. ^{1}H NMR spectra of **(a and a')** aminocellulose-grafted polycaprolactone (PCL-AC). (with permission from[2] Copyright © 2021 Elsevier Ltd.)

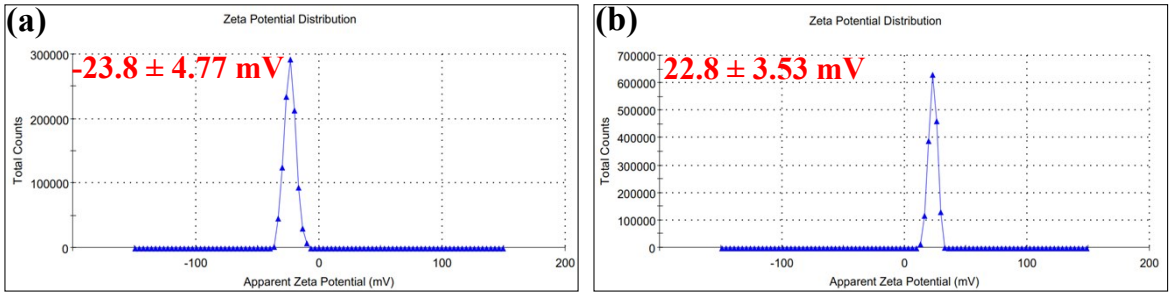


Fig. S4 Zeta potential analysis of (a) polycaprolactone (PCL) and (b) aminocellulose-grafted polycaprolactone (PCL-AC) by photon correlation spectroscopy. Experiments were repeated three times and data are reported as mean ± standard error of mean of three independent sets of observations (with permission from[2] Copyright © 2021 Elsevier Ltd.).

1.4. Formulation of nanoparticles

Core-shell nanoparticles were formulated with purpose of dual drug loading such that inner core part consists of gelatin nanoparticles loaded with hydrophilic glycyrrhizic acid (GA), while outer shell layer is composed of PCL-AC polymer for encapsulation of hydrophobic budesonide. Firstly, GA-loaded gelatin nanoparticles were formulated by completely dissolving gelatin and GA in water, followed by dropwise addition of equal volume of acetone. Acetone addition causes precipitation of GA-loaded gelatin nanoparticles, which were kept overnight on gentle stirring for solvent removal. Nanoparticles so formed were collected by centrifugation at 13000 rpm for 20 minutes and lyophilized.

These GA-loaded gelatin nanoparticles were then coated with PCL-AC, and co-loaded with budesonide in outer PCL-AC layer. GA-loaded gelatin nanoparticles were suspended in water containing 1% surfactant Pluronic F-127. In another addition funnel PCL-AC and budesonide were dissolved in dichloromethane and added dropwise to aqueous GA-loaded gelatin nanoparticle suspension with vigorous stirring. Here, PCL-AC along with budesonide gets coated over GA-loaded gelatin nanoparticles. Dual drug-loaded core-shell nanoparticles were then separated by centrifugation and lyophilized.

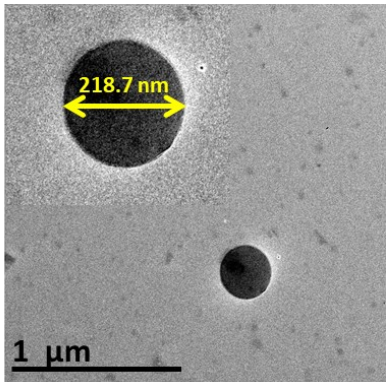


Figure S5. Representative photomicrograph for morphological characterization of uncoated glycyrrhizic acid loaded gelatin NPs with transmission electron microscopy (inset shows the TEM diameter of uncoated particles).

Table ST1. Stability study of NPs after 28 days

Stability parameters	Values
Particle size	244.3 ± 19
Zeta potential	30.10 ± 2.6
Polydispersity Index	0.29

2. Ultraviolet-visible (UV-Vis) spectroscopy

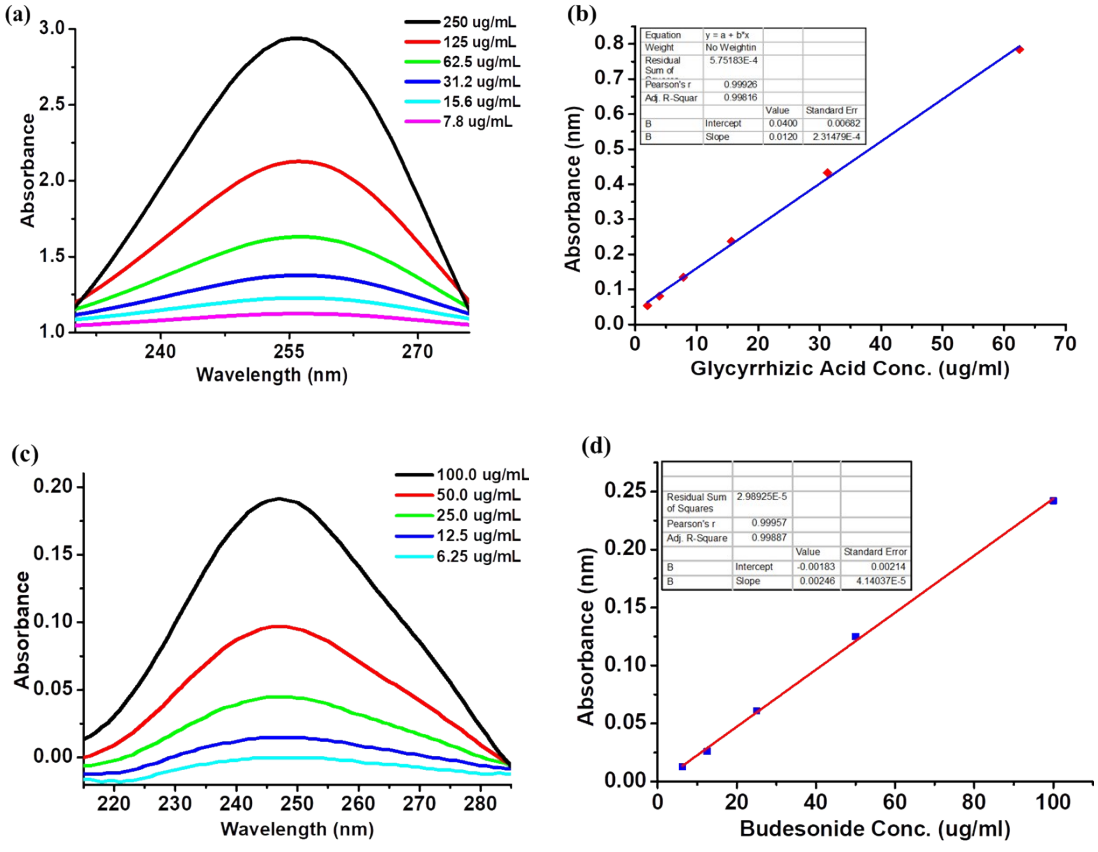


Fig. S6. UV-Vis spectral scans and standard calibration curves of GA and budesonide. (a) UV-Vis scan of GA at serially diluted different concentrations at its λ_{max}, (b) Calibration curve of GA for estimation of drug loading capacity and encapsulation efficiency, (c) UV-Vis scans of serially diluted different concentrations of budesonide at its λ_{max}, and (d) Calibration curve of budesonide for estimation of drug loading capacity and encapsulation efficiency[2].

3. Cytocompatibility and cytotoxicity of nanoparticles *in-vitro*

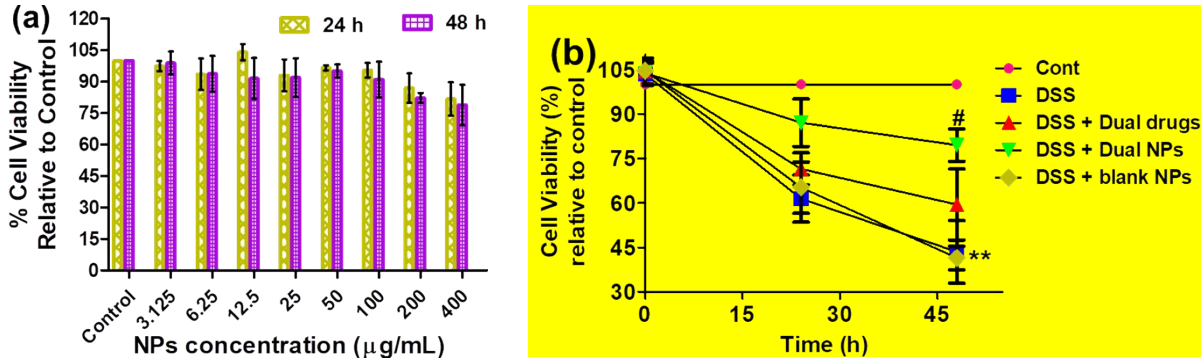


Fig. S7. (a) MTT assay for cytocompatibility of PCL-AC-gel NPs towards normal human foreskin fibroblasts (hTERT-BJ) cells. (b) Cytotoxicity of free drugs, blank NPs and dual drug loaded NPs in caco-2 cells. Experiments were performed in triplicate (n=3) and data are presented as mean ± standard deviation of three independent sets of observations[2].

4. Assessment of inflammatory mediators *in-vitro*

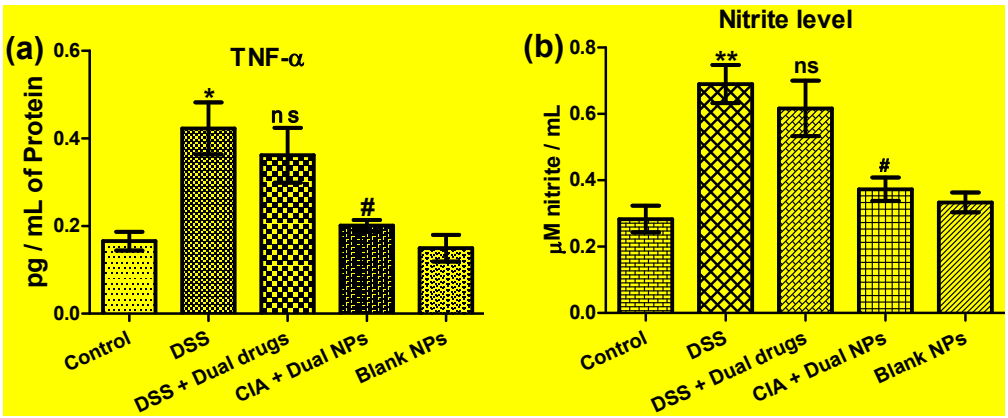


Figure S8. *In*-vitro effect of dual drugs loaded PCL-AC-gel NPs on **(a)** TNF-α level and **(b)** nitrite level in Cacp-2 cells. Significant differences are indicated by *p≤0.05, **p≤0.01, compared to control and #p≤0.05 as compared to DSS group. Experiments were performed in triplicate (n=3) and data are presented as mean ± SEM of three independent sets of observations.

5. Treatment regimen in mice

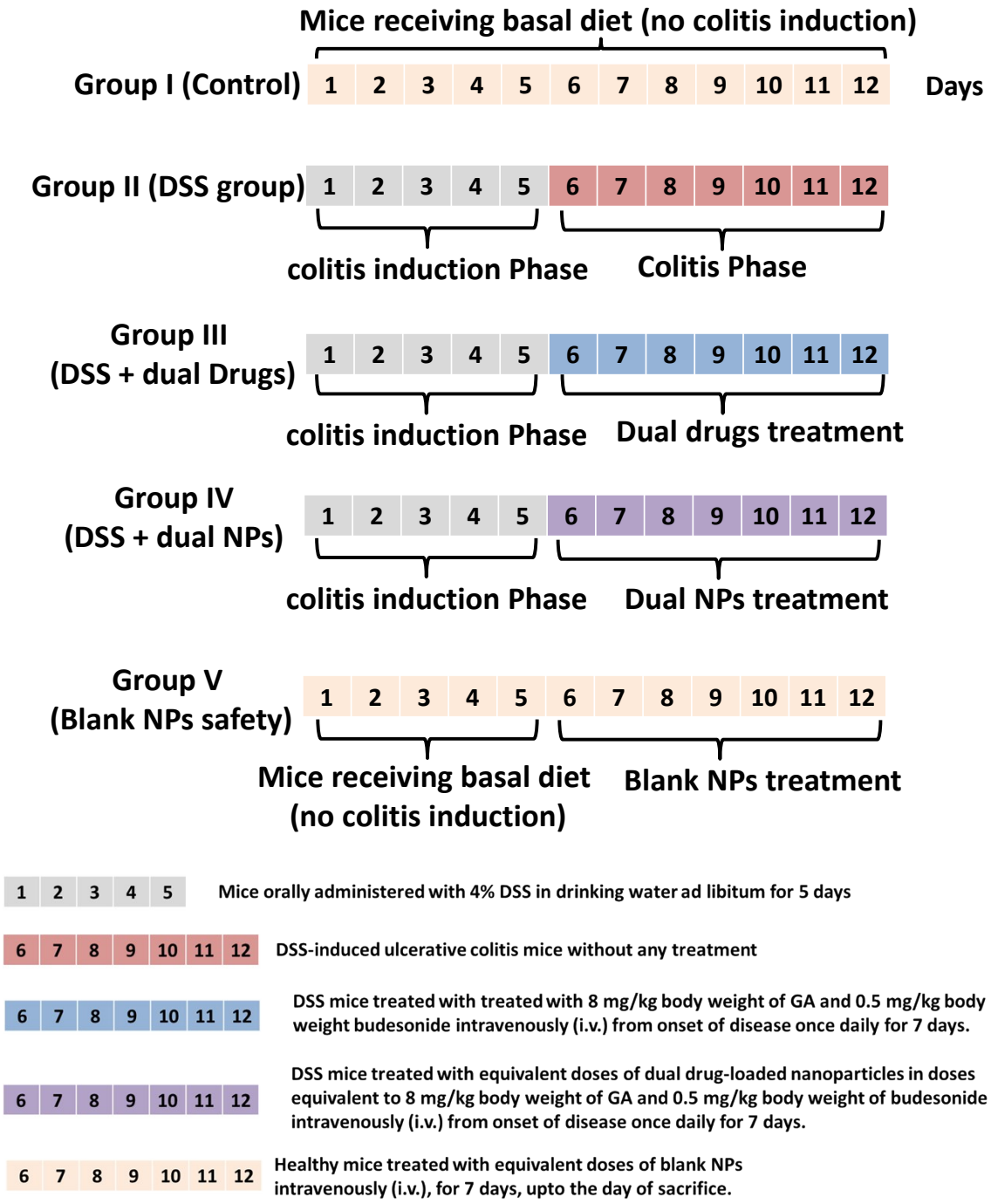


Figure S9. Schematic representation of the animal experimentation protocol.

6. Measurement of Clinical Severity of Colitis (Disease activity Index)

It includes three different physical parameters associated with colitis. These are physical activity, stool consistency and fecal occult bleeding. Physical activity has been scored on the basis of observation of experimental mice (0-normal or highly active, 1-mild active, 2-low active and 3-sedentary). Stool consistency was observed and scored (0-normal pellet, 1-soft,

2-loose watery stool, 3-stickiness, 4-diarrhoea). Similarly, after the induction of colitis rectal bleeding of each group has been observed and scored (0-no bleeding and 1-bleeding).

Table ST2. Average disease activity index scores among different groups of mice.

S. No.	Group	Disease activity index scores
1.	Control	0
2.	DSS	3.73 ± 0.4**
3.	DSS + Dual drugs	2.91 ± 0.5$^{\#}$
4.	DSS + Dual NPs	0.49 ± 0.3$^{\#\#}$
5.	Blank NPs	0

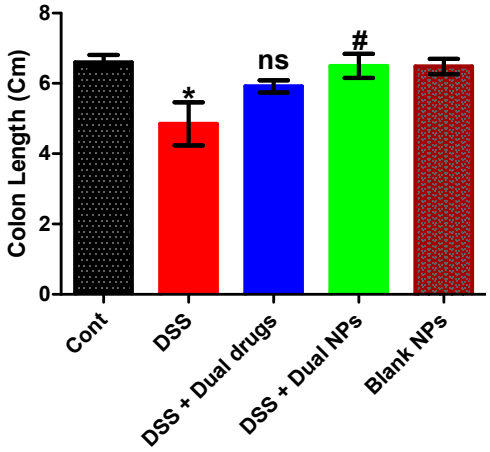


Figure S10. Semiquantitative analysis of colon length in mice of different groups. **Cont.** – Normal Control group showed normal colon length. **DSS:** mice treated with DSS showed the significant (*p≤0.05) reduction in colon length. **DSS + dual drugs:** DSS induced colitis mice treated with dual drugs showed non-significant (n.s.) restored colon length. **DSS + dual NPs:** DSS induced colitis mice treated with dual drug loaded NPs showed quite significant (#p≤0.05) restoration of colon length. **Blank NPs:** Normal mice treated with blank NPs showed normal colon length similar to that of control.

7. Immunohistochemical observations

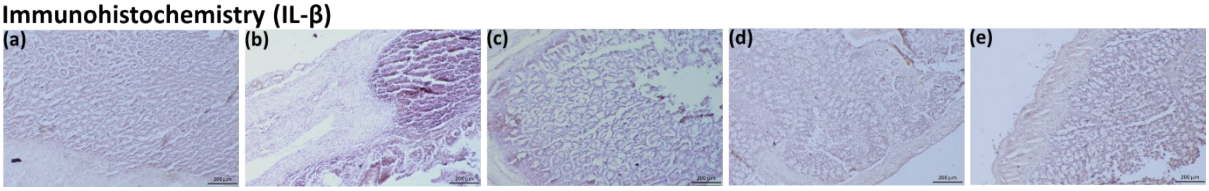


Figure S11. Representative photomicrographs of IL-1β expression in colon sections of mice. For immunohistochemical analysis, brown colour indicates specific immunostaining of IL-1β. (a) Normal Control animas. (b) DSS treated mice showing intense expression of IL-1β compared to control. (c) DSS + dual drugs treated group showing slightly less expression of IL-1β compared to DSS. (d) DSS + dual NPs group showing significantly lesser IL-1β expression compared to DSS and dual drugs. (e) Blank nanoparticles with no expression of IL-1β. Original magnification 20X. Scale bar = 50 µm. Experiments were performed in triplicate sections (n=3). N = 6 i.e. number of animals taken in each group.

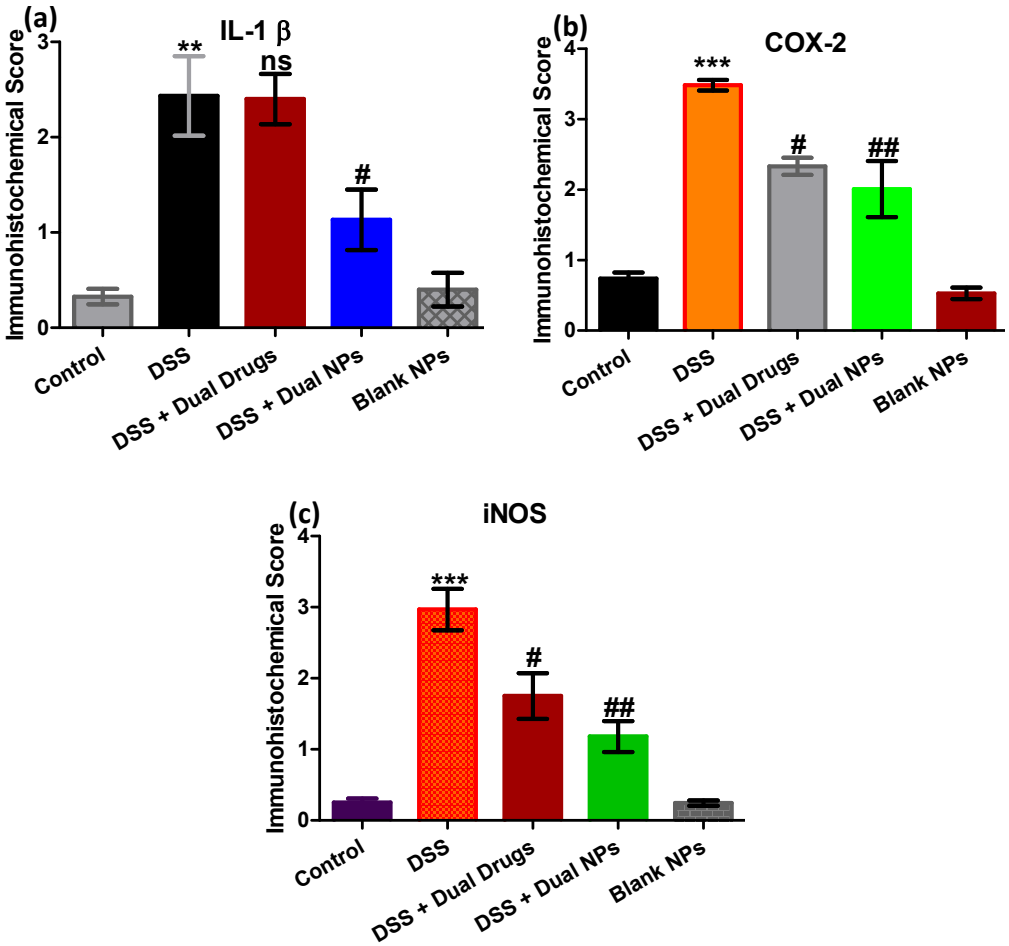


Figure S12. Semi-quantitative analysis of immunohistochemical expression of (a) IL1-β (b) COX-2 and (c) iNOS in colon sections of mice. Significant differences were indicated by **p≤0.01, ***p≤0.001, as compared to control and $^{\#}$p≤0.05 and $^{\#\#}$p≤0.01 as compared to DSS group. Data are presented as mean ± s.d. and in all datasets, n=3 i.e. colon tissues of different mice within same group.

9. Assessment of the bioavailability of drugs

10. Organ toxicity study

Assay for serum AST, ALT, BUN and creatinine

Nanoparticles were suspended in normal saline (N.S.) and vortexed vigorously to prevent NPs settling down or aggregation. 100 µL of this NP dispersion was administered to mice through tail vein. Blood was withdrawn via tail vein and serum parameters were measured using Vitros® 5600 integrated System.[3]

Table ST3. Serum parameters for safety of nanoparticles in liver and kidney of mice.

Parameters	**Groups**	
	Control	**Blank NPs**
AST (IU/L)	216.7 ± 10.14	220.91 ± 13.3
ALT (IU/L)	240 ± 11.1	247.1 ± 9.85
Creatinine (mg/dl)	0.12 ± 0.02	0.11 ± 0.03
BUN (mg/dl)	19.33 ± 2.85	18.74 ± 1.97

References

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