Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

Supplementary Information

Porous ZnO nanorod for targeted delivery of doxorubicin: *in vitro* and *in vivo* response for therapeutic applications

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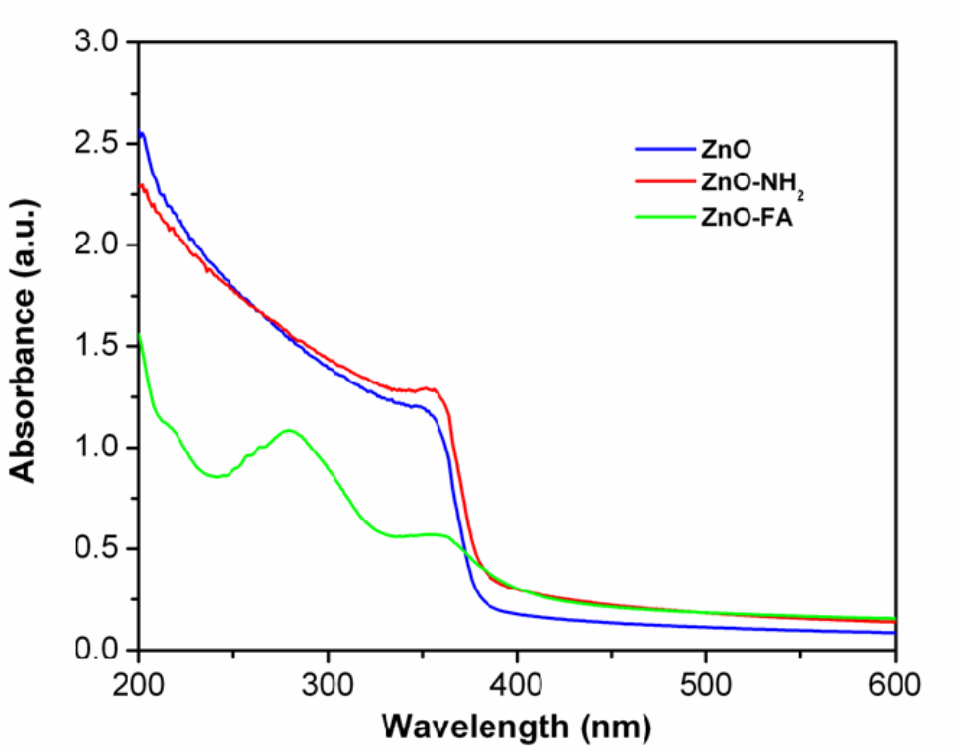

Figure S1. UV-Vis absorbance spectra of ZnO, ZnO-NH2 and ZnO-FA respectively.

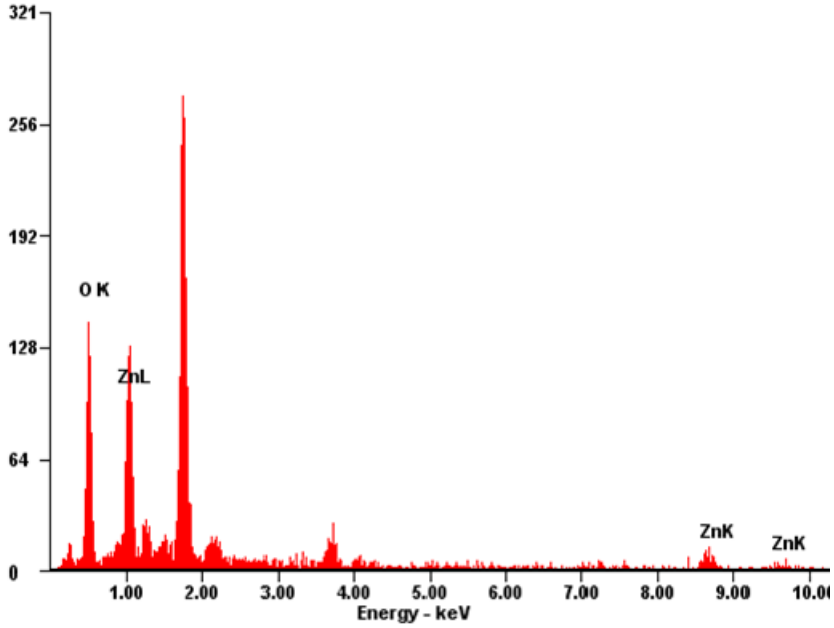

Figure S2. EDX spectrum of ZnO, which justified Zn and O were the main chemical components.

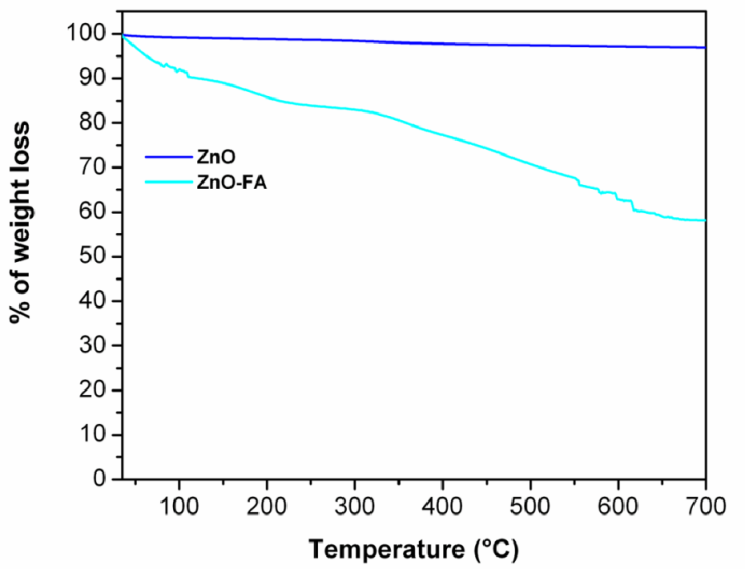

Figure S3. TG profile indicating % of weight loss of ZnO and ZnO-FA against temperature respectively.

Only ZnO underwent only 3% of weight loss throughout the temperature range due to the loss of water molecules. While ZnO-FA underwent weight loss up to 110°C due to the evaporation of water molecules, weight loss above 150°C and 250°C was attributed to the loss of 3-aminopropylphosphonic acid and folic acid fragment.

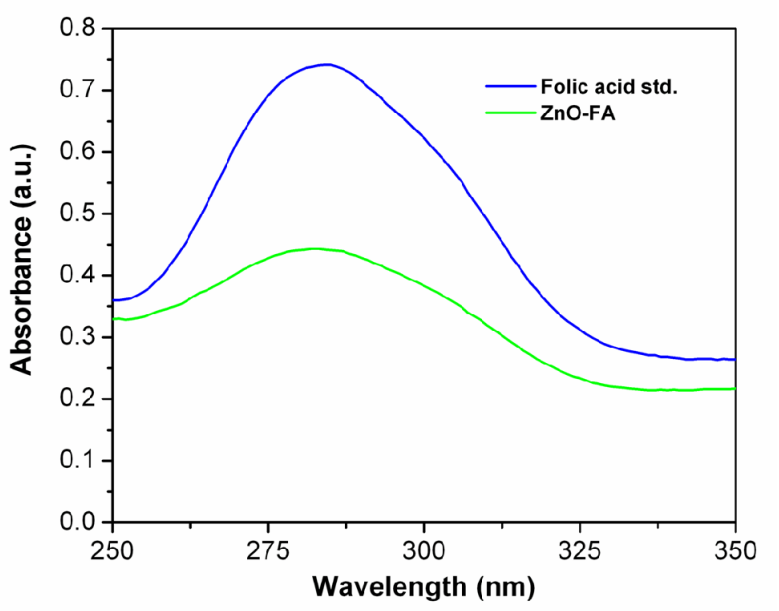

Figure S4. UV-Vis absorbance justifying calibration curve of standard folic acid against ZnO-FA dispersion.

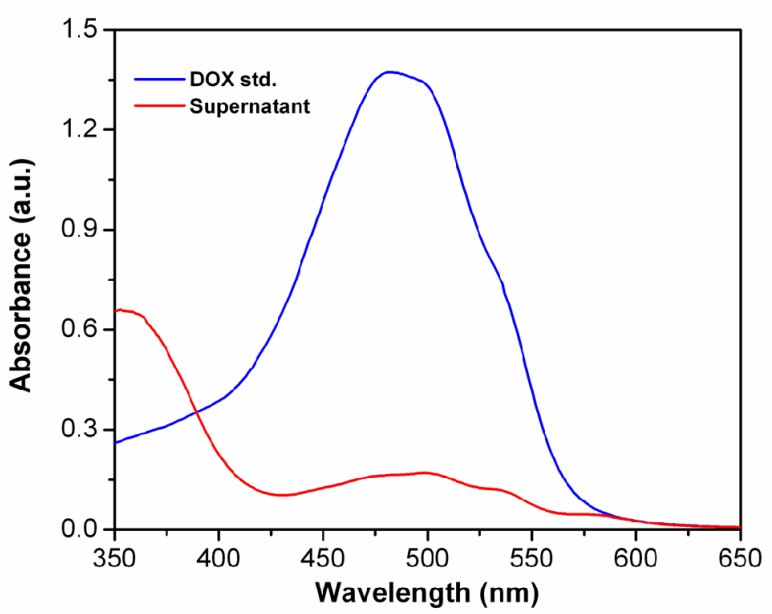

Figure S5. UV-Vis absorbance of standard DOX solution and the supernatant obtained after treatment with ZnO-FA.

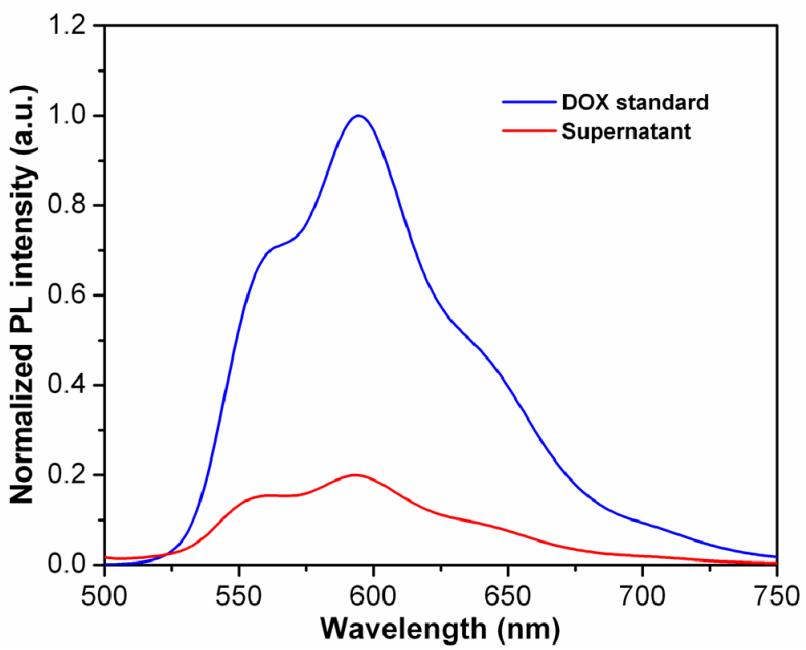

Figure S6. PL spectra of standard DOX solution and the supernatant obtained after treatment with ZnO-FA.

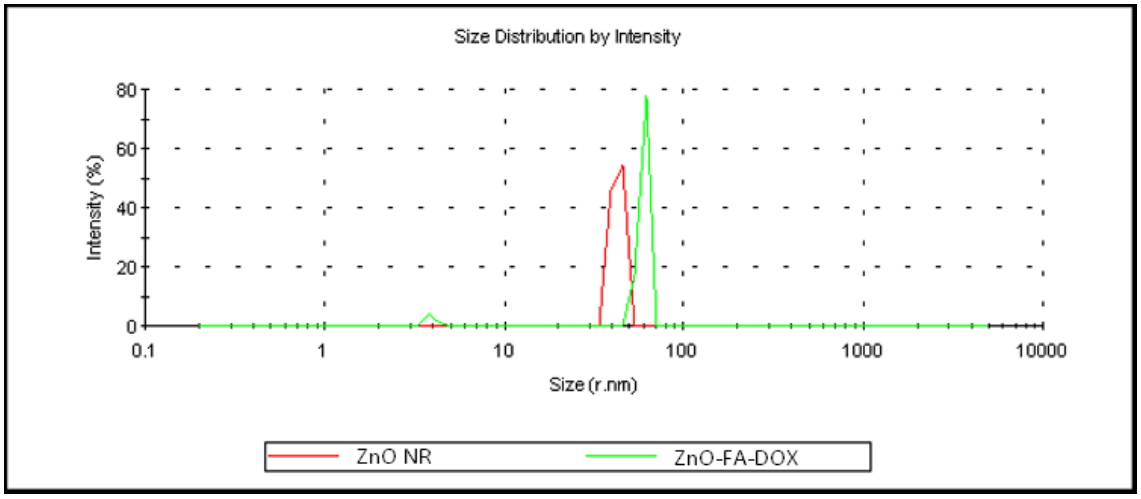

Figure S7. A comparison of average hydrodynamic radius of ZnO nanorod and ZnO-FA-DOX.

ZnO nanorod exhibited an average hydrodynamic radius of around 60 nm which was increased marginally after its functionalization followed by conjugation with folic acid and DOX loading as expected. However it remained well dispersed in the medium.

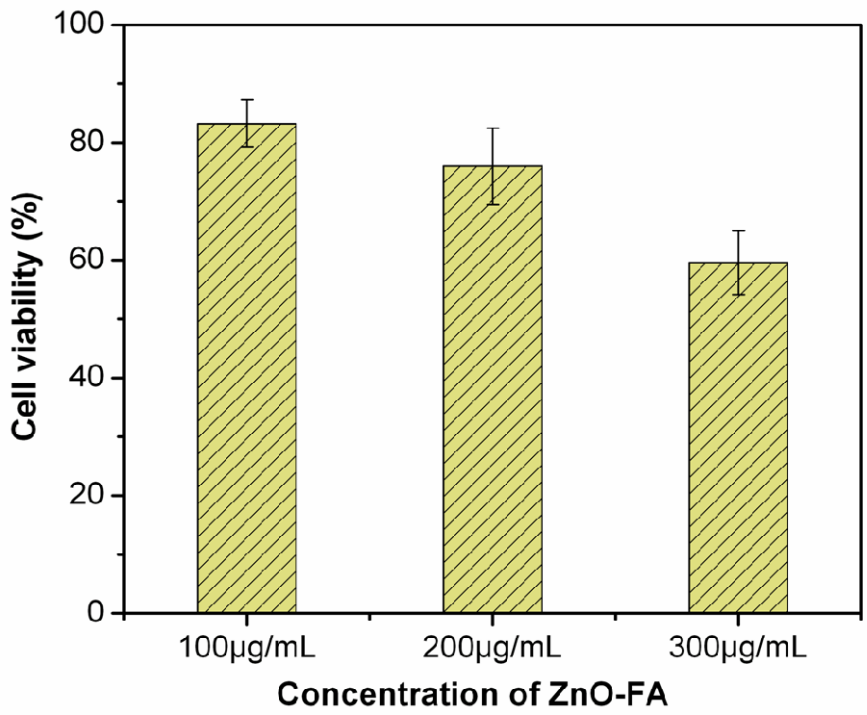

Figure S8. MTT assay showing the cellular viability of MDA-MB-231 cells against three different concentrations of ZnO-FA.

MTT assay on MDA-MB-231 cells also justified that only ZnO-FA was fairly nontoxic in this case as well, nearly 83% viable cells were present at a concentration of 100 µg/mL of ZnO-FA and ~60% cells were alive at 300 µg/mL of ZnO-FA treatment.

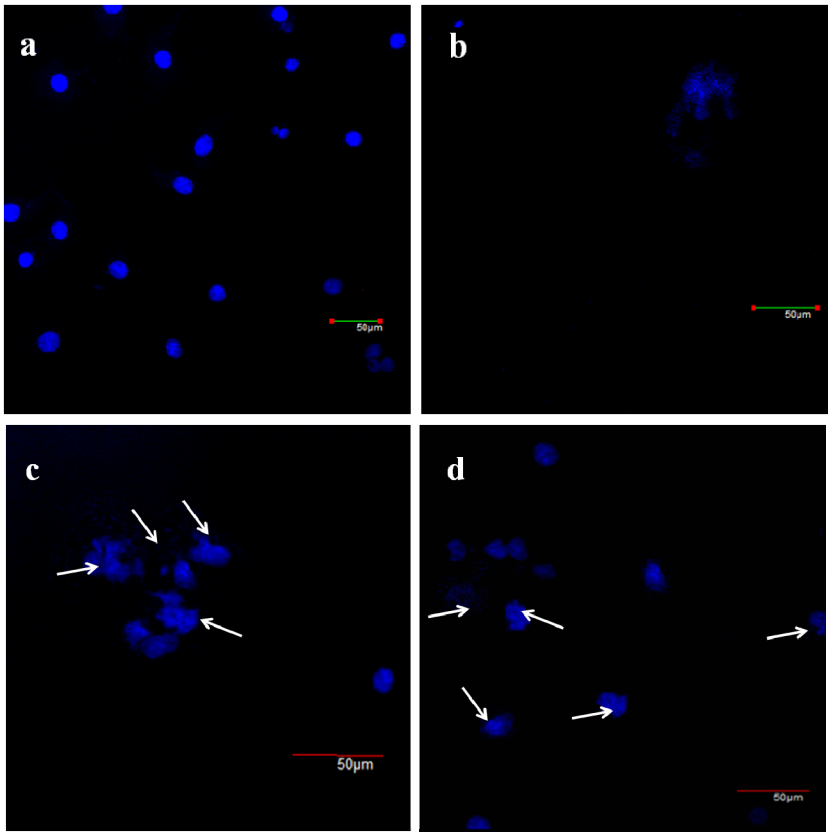

Figure S9. Apoptosis study of nuclear fragmentation after 24h by DAPI staining. (a) Control MDA-MB-231 cell exhibiting no alterations in nuclear morphology, (b) 1 µg/mL ZnO-FA-DOX, (c) 10 µg/mL ZnO-FA-DOX and (d) 10 µg/mL of DOX treated MDA-MB-231 cells after 24h; where ZnO-FA-DOX and DOX exhibited significant rupture in nuclear morphology. Arrows in the last two images signified rupture in nuclear morphology where similar concentration of DOX was used as a control.

Table T1. A comparison of blood biochemical parameters between control and ZnO treated mice.

Parameters		Control	ZnO		
			100 µg/mL	200 µg/mL	300 µg/mL
Haemoglobin (g/dL)		14.76 ± 0.057735	14.8 ±0.1	14.4 ±0.1	14.3 ±0.1
TC	RBC (million/ mm^3)	4.9 ±0.1	4.86 ±0.057735	4.73±0.057735	4.56±0.057735
	WBC/ mm^3	4222.6 ±20.5264	4218±15.874	4211.6±29.297	4205±22.479
DC (%)	Neutrophils	37.66±0.5777	40±1	41±1	40±1
	Lymphocytes	58±1	58±1	56±1	55±1
	Monocytes	02±0	02±0	02±0	02±0
	Eosinophils	02±0	02±0	02±0	02±0
	Basophils	0±0	0±0	0±0	0±0
Platelets (lakh/ mm^3)		1.51±0.1	1.496±0.00577	1.513±0.00577	1.526±0.00577
LDH (IU/L)		217.33±1.1547	217.66±1.1547	220.33±1.1547	223.33±1.1547
Creatinine (mg/dL)		0.813±0.00577	0.76±0.01154	0.77±0.00577	0.806±0.00577
Alkaline Phosphate (IU/L)		60.33±0.577	62.33±0.577	65.66±0.577	71±1.73
Total Protein (gm/dL)		6.93±0.0577	6.96±0.0577	6.86±0.0577	6.96±0.0577
Cholesterol (mg/dL)		115.6±1.527	119±1	120±1	120±1
Triglyceride (mg/dL)		71.33±1.154	75±1	76.33±0.577	76.33±0.577
Uric Acid (mg/dL)		5.0±0.1	5.06±0.0577	5.13±0.0577	4.93±0.0577
BUN (mg/dL)		13.96±0.0577	12±1	13.33±0.577	13.33±0.577
SGOT (IU/L)		14.03±0.0577	17.33±0.577	19.33±1.154	22±1
SGPT (IU/L)		12.03±0.0577	14.33±0.577	14.66±1.154	13.33±0.577
Phosphorous (mg/dL)		3.403±0.005	3.16±0.01154	3.11±0.01154	3.093±0.00577

Table T2. A comparison of blood biochemical parameters between control and ZnO-FA treated mice.

Parameters		Control	ZnO-FA		
			100 µg/mL	200 µg/mL	300 µg/mL
Haemoglobin (g/dL)		14.76 ± 0.057735	15 ±0.1	14.9 ±0.1	15 ±0.1
TC	RBC (million/ mm^3)	4.9 ±0.1	4.93 ±0.057735	4.86±0.057735	5.03±0.057735
	WBC/ mm^3	4222.6 ±20.5264	4218±23.065	4216±28.867	4225.3±22.030
DC (%)	Neutrophils	37.66±0.5777	42.66±0.577	35.66±0.577	39±1
	Lymphocytes	58±1	59±1	58.66±1.154	57.66±0.577
	Monocytes	02±0	02±0	02±0	02±0
	Eosinophils	02±0	02±0	02±0	02±0
	Basophils	0±0	0±0	0±0	0±0
Platelets (lakh/ mm^3)		1.51±0.1	1.516±0.00577	1.53±0.01	1.536±0.0115
LDH (IU/L)		217.33±1.1547	217.66±1.1547	219.66±1.1547	221.33±1.1547
Creatinine (mg/dL)		0.813±0.00577	0.823±0.00577	0.84±0.01	0.84±0.01
Alkaline Phosphate (IU/L)		60.33±0.577	62±1	62.33±0.577	66±1
Total Protein (gm/dL)		6.93±0.0577	6.63±0.0577	6.73±0.0577	6.96±0.0577
Cholesterol (mg/dL)		115.6±1.527	118±1	118±1	119±1
Triglyceride (mg/dL)		71.33±1.154	74±1	73.66±0.577	74±1
Uric Acid (mg/dL)		5.0±0.1	4.76±0.0577	4.83±0.0577	4.76±0.0577
BUN (mg/dL)		13.96±0.0577	12±1	12.66±0.577	13.33±0.577
SGOT (IU/L)		14.03±0.0577	16.33±0.577	20.66±0.577	21±1
SGPT (IU/L)		12.03±0.0577	12.66±0.577	12.66±0.577	11.66±0.577
Phosphorous (mg/dL)		3.403±0.005	3.01±0.01154	3.13±0.01154	3.15±0.0115

Table T3. Comparison of blood biochemical parameters between control and ZnO-FA treated mice by i.v. injection.

Parameters		Treatment		
		Control	ZnO-FA 300 µg/mL after 24h	ZnO-FA 300 µg/mL after 7 days
Haemoglobin (g/dL)		14.53± 0.11547	14.3 ±0.1	14.73 ±0.0577
TC	RBC (million/ mm^3)	4.83 ±0.057	4.86±0.057735	4.8 ±0.1
	WBC/ mm^3	4250 ±45.825	4220±26.45	4213.33±20.82
DC (%)	Neutrophils	37±1	37.33±1.154	38±1.732
	Lymphocytes	62±1	65.33±3.05	65.33±1.154
	Monocytes	02±0	02±0	02±0
	Eosinophils	02±0	02±0	02±0
	Basophils	0±0	0±0	0±0
Platelets (lakh/ mm^3)		1.51±0.1	1.516±0.00577	1.543±0.00577
LDH (IU/L)		218.33±0.577	222±2	222.66±2.309
Creatinine (mg/dL)		0.766±0.011547	0.713±0.01154	0.74±0.01
Alkaline Phosphate (IU/L)		64±2	72±1	73.66±0.577
Total Protein (gm/dL)		6.83±0.0577	6.5±0.2645	6.73±0.0577
Cholesterol (mg/dL)		116±2	118.33±2.081	119±1.73
Triglyceride (mg/dL)		70±2	72.66±0.577	73.66±0.577
Uric Acid (mg/dL)		5.0±0.1	4.9±0.1	4.9±0.1
BUN (mg/dL)		13.63±0.152	13.13±0.11547	13.26±0.305
SGOT (IU/L)		14.1±0.1	16.2±0.2	16.43±0.404
SGPT (IU/L)		12.36±0.2516	13.06±0.11547	13.03±0.152
Phosphorous (mg/dL)		3.4±0.00577	3.413±0.01154	3.406±0.01154

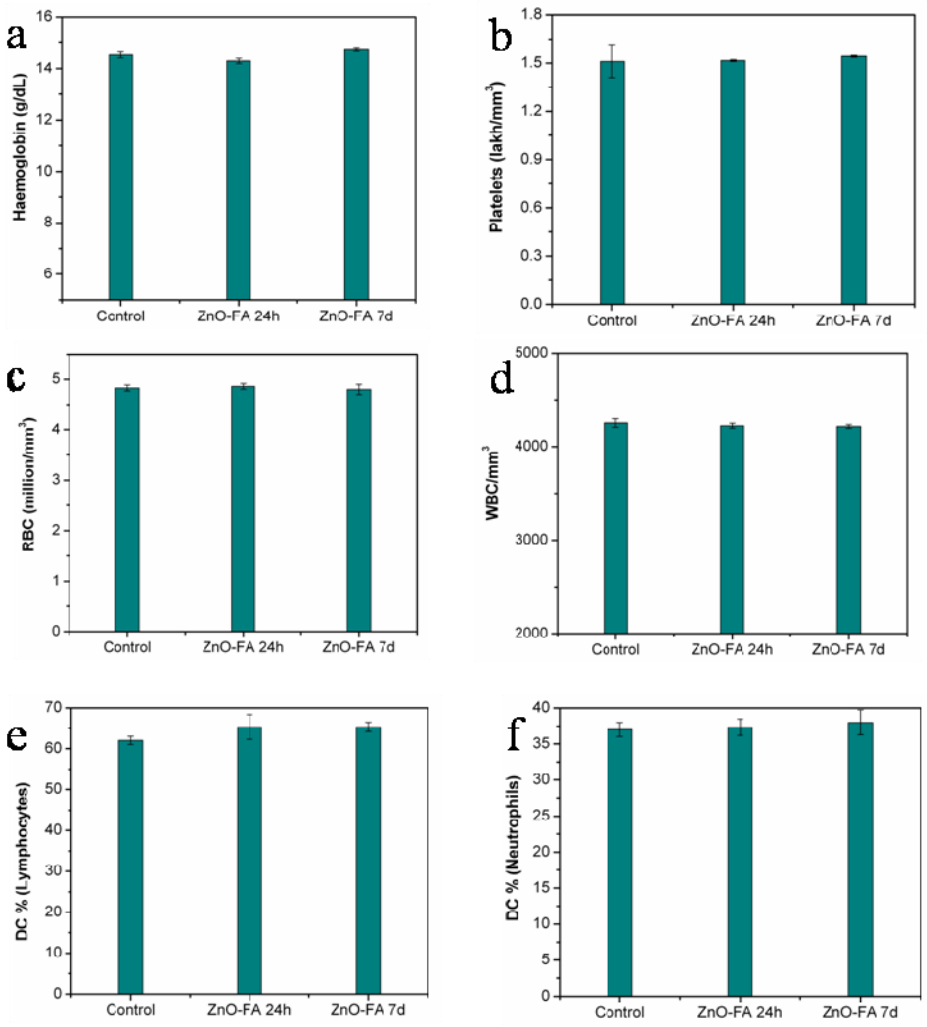

Figure S10A. Diagrammatic representation of relative hematology indicators such as (a) Haemoglobin, (b) Platelets count, (c) Red blood cell count (RBC), (d) White blood cell count (WBC), (e) Lymphocytes and (f) Neutrophils of Control samples, ZnO-FA treated mice after 24h (intravenous injection) and ZnO-FA treated mice after 7d (intravenous injection). Systematic toxicity study on hematology parameters suggested that no significant toxicity or any significant changes were observed in post intravenous injected mice, therefore ZnO-FA was biocompatible.

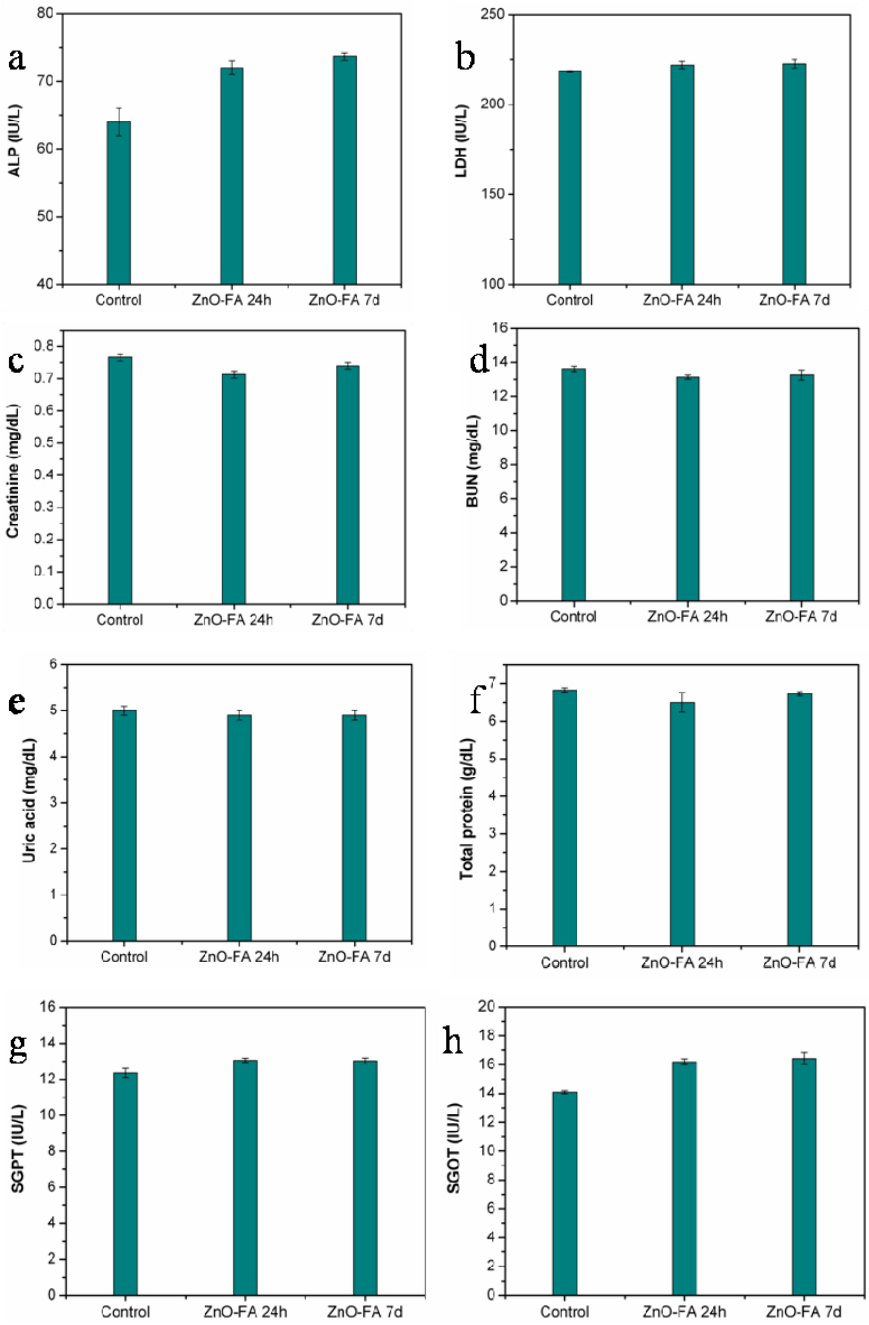

Figure S10B. Diagrammatic representation of relative serum biochemical indicators such as (a) Alkaline phosphatase (ALP), (b) Lactate dehydrogenase (LDH), (c) Creatinine, (d) Blood urea nitrogen (BUN), (e) Uric acid, (f) Total protein, (g) Serum glutamic pyruvic transaminase (SGPT) and (h) Serum glutamic oxaloacetic acid (SGOT) of Control samples, ZnO-FA treated mice after 24h (intravenous injected) and ZnO-FA treated mice (intravenous injected) after 7d. Systematic toxicity study on serum biochemical parameters suggested that no significant toxicity or any significant changes were observed in post intravenous injected mice except for ALP. Treated mice exhibited slight raise in ALP values.

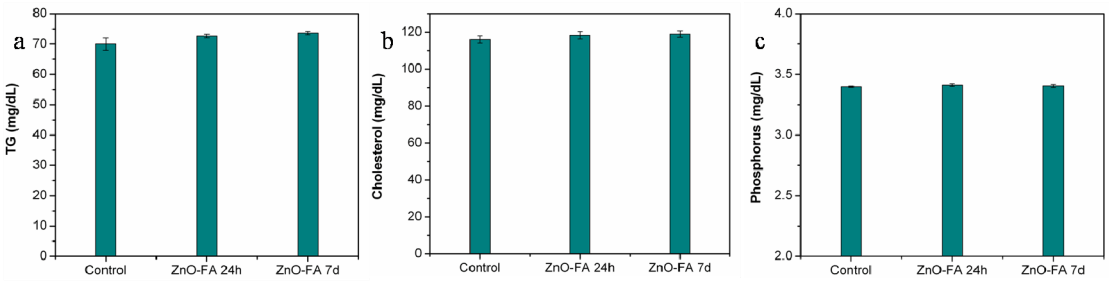

Figure S10C. Diagrammatic representation of other biochemical parameters such as (a) Triglyceride (TG), (b) Cholesterol and (c) Phosphorus of Control samples, ZnO-FA treated mice after 24h (intravenous injected) and ZnO-FA treated mice after 7d (intravenous injected). No significant toxicity was observed in all the above cases of ZnO-FA by intravenous injection pathway. Therefore oral administration route as well as intravenous injection route justified biocompatibility of ZnO-FA.

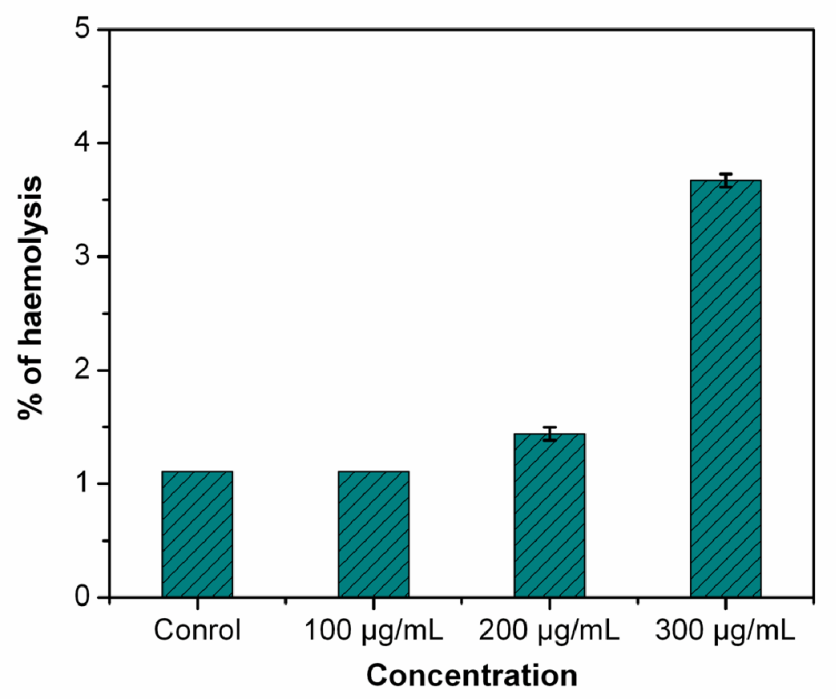

Figure S11. Representation of *in vitro* haemolysis on human RBC.

In vitro haemolysis experiment justified that no significant lysis was observed even after treatment with ZnO-FA. In control set only 1.11% haemolysis was observed; while on the treated set (with maximum concentration of ZnO-FA 300 µg/mL) negligible haemolysis of around 3.67% was observed which was again comparable with the control ones. Therefore ZnO-FA have no adverse effect on human RBC.

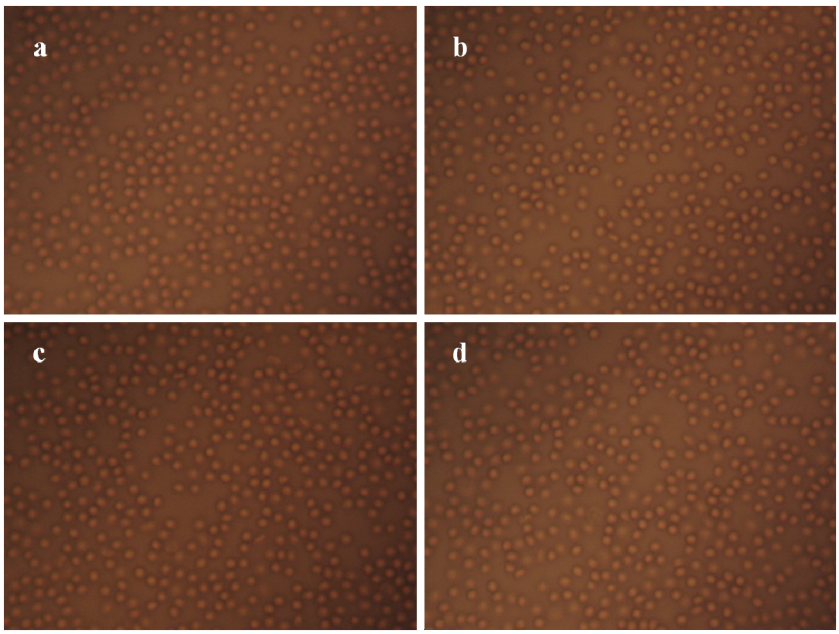

Figure S12. Light microscopic image of Human RBC; (a) Control (without ZnO-FA treatment), (b) 100 µg/mL of ZnO-FA treated, (c) 200 µg/mL of ZnO-FA treated, (d) 300 µg/mL of ZnO-FA treated.

Light microscopic image showed that no rupture or alteration in morphology was observed in all the three treated sets with respect to the control one. Healthy intact RBC was observed in all the cases.

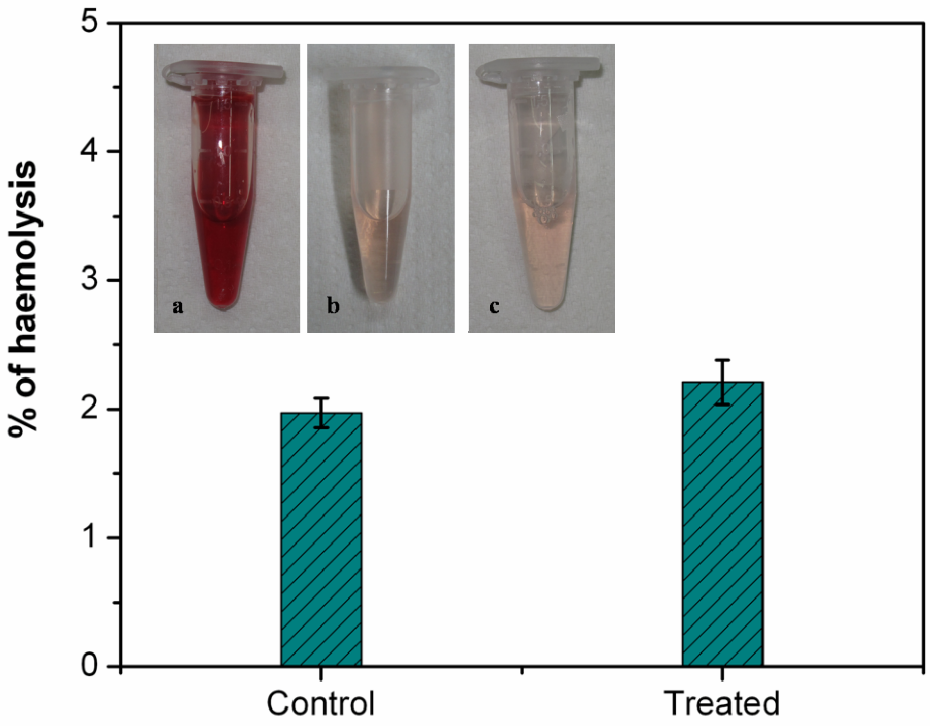

Figure S13. *In vivo* haemolysis experiment on mice model. The mice were intravenous injected with 100µL ZnO-FA of concentration 300 µg/mL through tail vein. After 1h of injection the blood was isolated and haemolysis measurement was carried out. Interestingly no significant haemolysis was observed after intravenous injection; only 2.2% haemolysis was observed in the treated one while the control one showed ~2% of haemolysis. The inset showed digital image of supernatant during experiment (a) 100% haemolysis (in presence of water), (b) Control set and (c) 300 µg/mL ZnO-FA treated set. The digital image also justified that no significant difference was present in between the control and treated set.

A brief literature review of ZnO based nanostructures in drug delivery systems.

SL. No.	**Nature of ZnO**	**Surface area**	**Application in TDD**	***In vitro* / *In vivo* applications**	**Reference**
1	Mesoporous 3D assembly	27.5 m^2/g (S_{BJH})	Not targeted delivery	*In vitro*	J. Mater. Chem., 2010, 20, 6446.
2	Mg doped ZnO quantum dots	–	Targeted delivery	*In vitro*	J. Mater. Chem., 2011, 21, 13406.
3	ZnO nanorods	–	Photodynamic therapy (PDT), not TDD	*In vitro*	Biomater., 2011, 32, 1906.
4	Chitosan encapsulated ZnO quantum dots	–	Targeted delivery	*In vitro*	Acta Biomater., 2010, 6, 2732.
5	ZnO nanoparticles	–	Not targeted delivery	*In vitro*	Nano Res., 2009, 2, 882.
6	Porous hexagonal ZnO	25 m^2/g (S_{BJH})	–	–	J. Phys. Chem. C, 2007, 111, 1113.
7	ZnO nanoporous pyramid	127.7 m^2/g (S_{BET})	–	–	J. Mater. Chem., 2012, 22, 6539.
8	**Porous ZnO nanorod**	**305.14 m^2/g (S_{BET})**	**Targeted delivery**	***In vitro* and *In vivo* application**	**This work.**

To best of our knowledge this is the first work on TDD using porous ZnO nanostructures with high surface area and *in vitro* and *in vivo* applications.