Supplementary materials

Supplementary table 1: Box–Behnken's design with actual and predicted values of particle size (PS), poly dispersity index (PDI) and entrapment efficiency of UFG loaded PLGA nanoparticles.

Run Order	Particle Size		PDI		EE(%)	
	Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value
1	278.00	324.53	0.24	0.35	78.60	75.71
2	260.00	239.60	0.24	0.20	84.40	82.07
3	254.00	287.89	0.30	0.31	66.20	62.75
4	218.00	205.65	0.23	0.24	83.58	84.59
5	190.00	184.18	0.21	0.20	86.20	86.56
6	283.00	291.77	0.23	0.24	72.90	75.95
7	333.20	337.29	0.36	0.34	80.60	79.24
8	304.20	304.92	0.40	0.36	76.48	76.19
9	270.00	311.94	0.29	0.35	62.90	62.79
10	409.00	393.27	0.43	0.43	58.30	60.38
11	202.00	179.74	0.16	0.18	84.98	87.71
12	190.00	184.18	0.21	0.20	86.20	86.56
13	187.00	156.69	0.16	0.14	90.64	90.03
14	524.00	463.89	0.59	0.54	48.50	49.98
15	190.00	184.18	0.21	0.20	86.20	86.56
16	190.00	184.18	0.21	0.20	86.20	86.56
17	208.00	256.47	0.25	0.29	84.10	83.34

Supplementary table 2: Analysis of variance of calculated model of UFG-PLGA Nanoparticle for particle size (PS), polydispersity index (PDI) and entrapment efficiency (EE).

	PS	PDI	EE
Regression			
Model	Quadratic model	Quadratic model	Quadratic model
Sum of square	1.192E+005	0.17	2189.53
Df	9	9	9
Mean squares	13241.42	0.019	243.28
F value	6.87	5.84	31.85*
P	0.0094	0.0149	< 0.0001
Residual			
Sum of square	13494.59	0.023	53.47
Df	7	7	7
Mean squares	1927.80	3.319E-003	7.64
Lack of fit test			
Sum of square	*13494.59*	*0.023*	*53.47*
Df	4	4	3
Mean squares	*3373.65*	*5.808E-003*	13.37
Coefficient correlation (r2)	0.8983	0.8824	0.9762
Coefficient of variation (CV%)	16.62	20.70	3.57
Adequate precision valuě*	9.123	9.022	18.893
Std Deviation	43.91	0.058	2.76
Adj R-Squared	0.7675	0.7312	0.9455

*"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 9.123 for PS , 9.022 for PDI and 18.893 for EE indicates an adequate signal which concludes that this model can be used to navigate the design space.

* The Model F-value of 31.85 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Supplementary table 3: Effect of UFG-PLGA-NP treatment on the development of macroscopic hepatocytes nodules induced by DEN in rats.

S. No	Groups	Number of rats/Number of rats with nodules	Nodules Incidence	Total Number of Nodules
1	DEN Control	7/7	95	202
2	DEN Control+UFG (5 mg/kg)	12/10	85	179
3	DEN Control+UFG (50 mg/kg)	12/7	68	122
4	DEN Control+UFG-PLGA-NP	12/3	39	69

Group I (Normal control) and Group II (Normal control+UFG 50 mg/kg) did not show any visible hepatocytes nodule.

Supplementary table 4: Effect of UFG-PLGA-NP treatment on the development of the macroscopic tumor induced by DEN in rats.

| S. No	Groups	Number of rats/Number of rats with tumor	Tumor incidence (%)
1	DEN Control	7/7	100
2	DEN Control+UFG (5 mg/kg)	12/10	83.33
3	DEN Control+UFG (50 mg/kg)	12/7	58.33
4	DEN Control+UFG-PLGA-NP	12/3	25

Group I (Normal control) and Group II (Normal control+UFG 50 mg/kg) did not show any visible hepatocytes nodule.
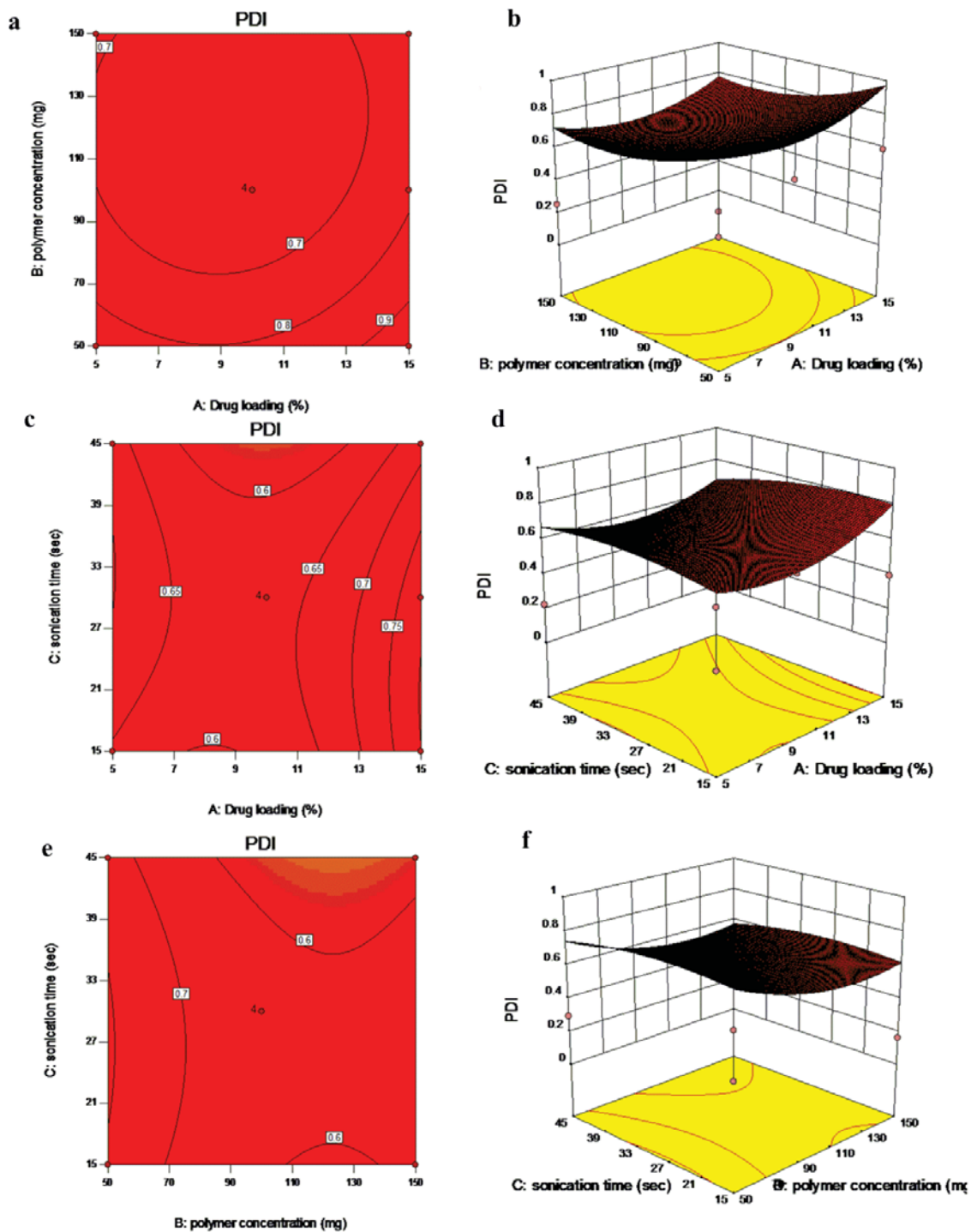
Supplementary figure 1: Three-dimensional (3D) response surfaces (B, D and F) and contour plots (A, C and E) showing the effects of polymer concentration, drug percentage and sonication time on the Polydispersity index (PDI) of the Umbelliferone β-D-galactopyranoside (UFG) nanoformulation.
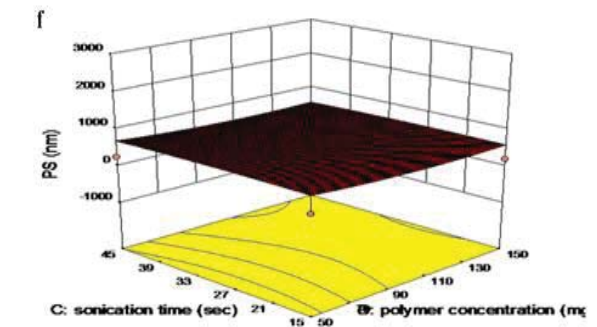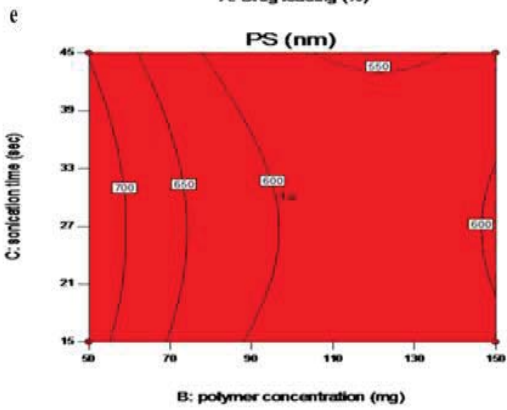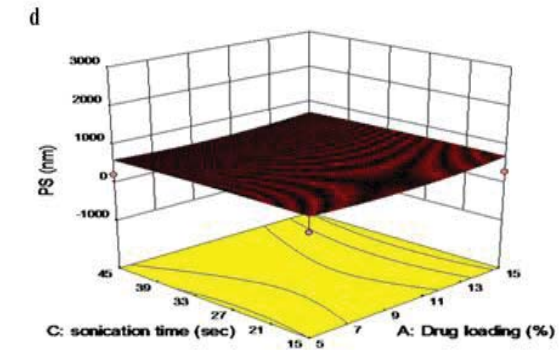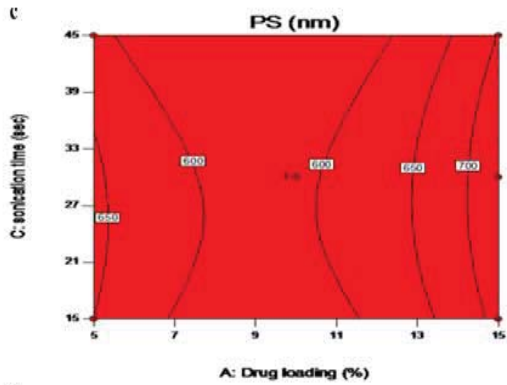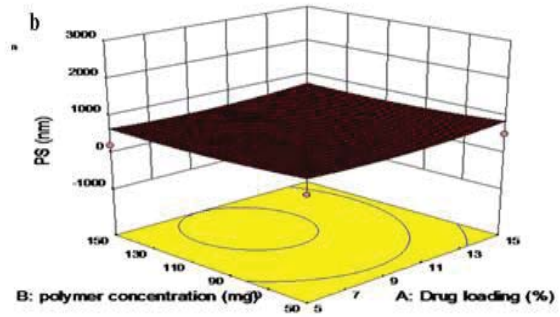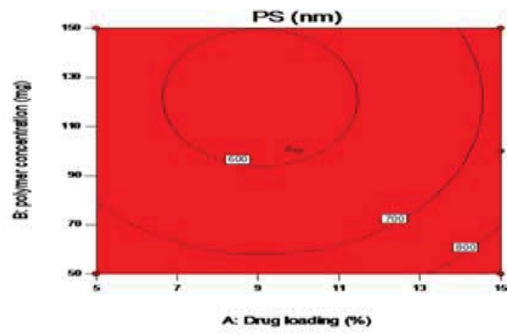
Supplementary figure 2: Three-dimensional (3D) response surfaces (B, D and F) and contour plots (A, C and E) showing the effects of polymer concentration, drug percentage and sonication time on the particle size (PS) of the Umbelliferone β-D-galactopyranoside (UFG) nanoformulation.
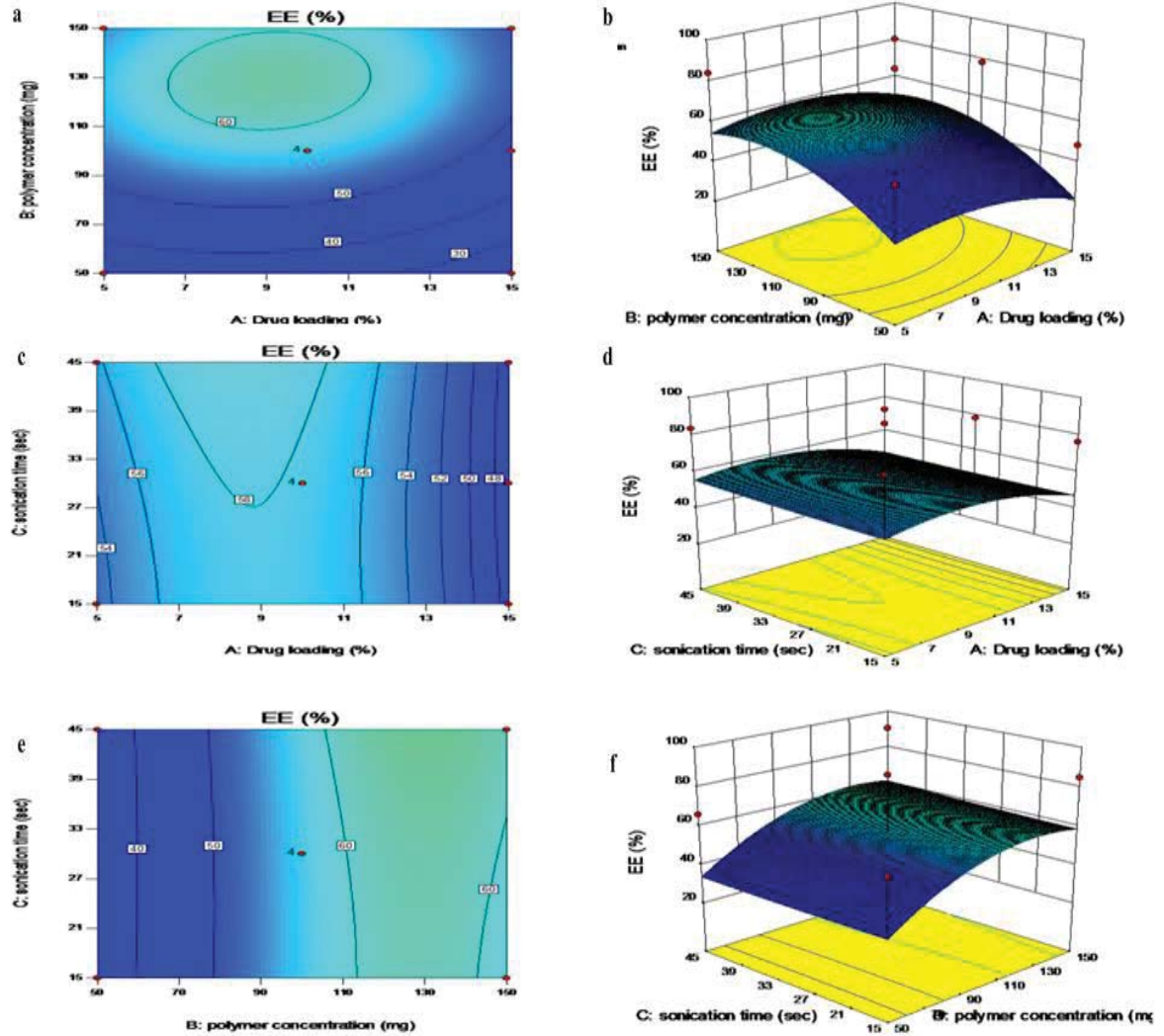
Supplementary figure 3: Three-dimensional (3D) response surfaces (B, D and F) and contour plots (A, C and E) showing the effects of polymer concentration, drug percentage and sonication time on the entrapment efficiency (EE) of the Umbelliferone β-D-galactopyranoside (UFG) nanoformulation.
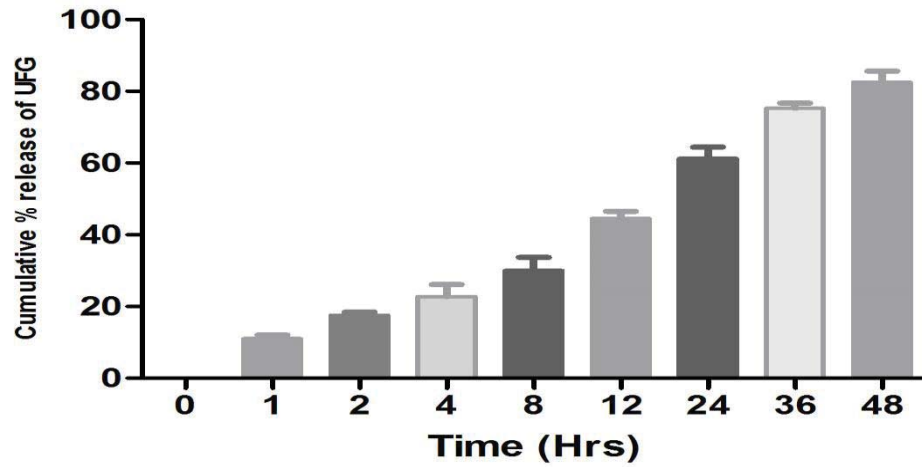
**Supplementary figure 4:** In vitro release of Umbelliferone β-D-galactopyranoside (UFG) from the polymeric nanoformulation as a function of time at different time intervals (0–48 h)using the dialysis method in phosphate buffer at pH 7.4.
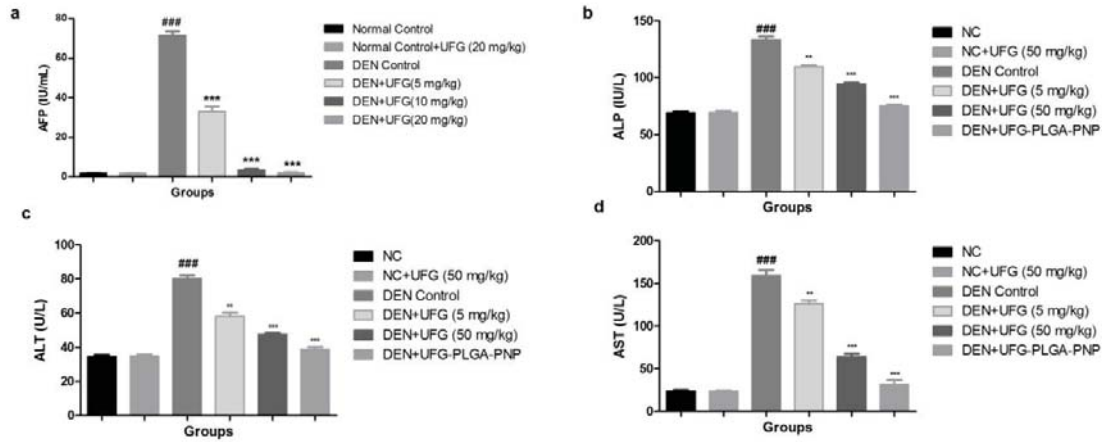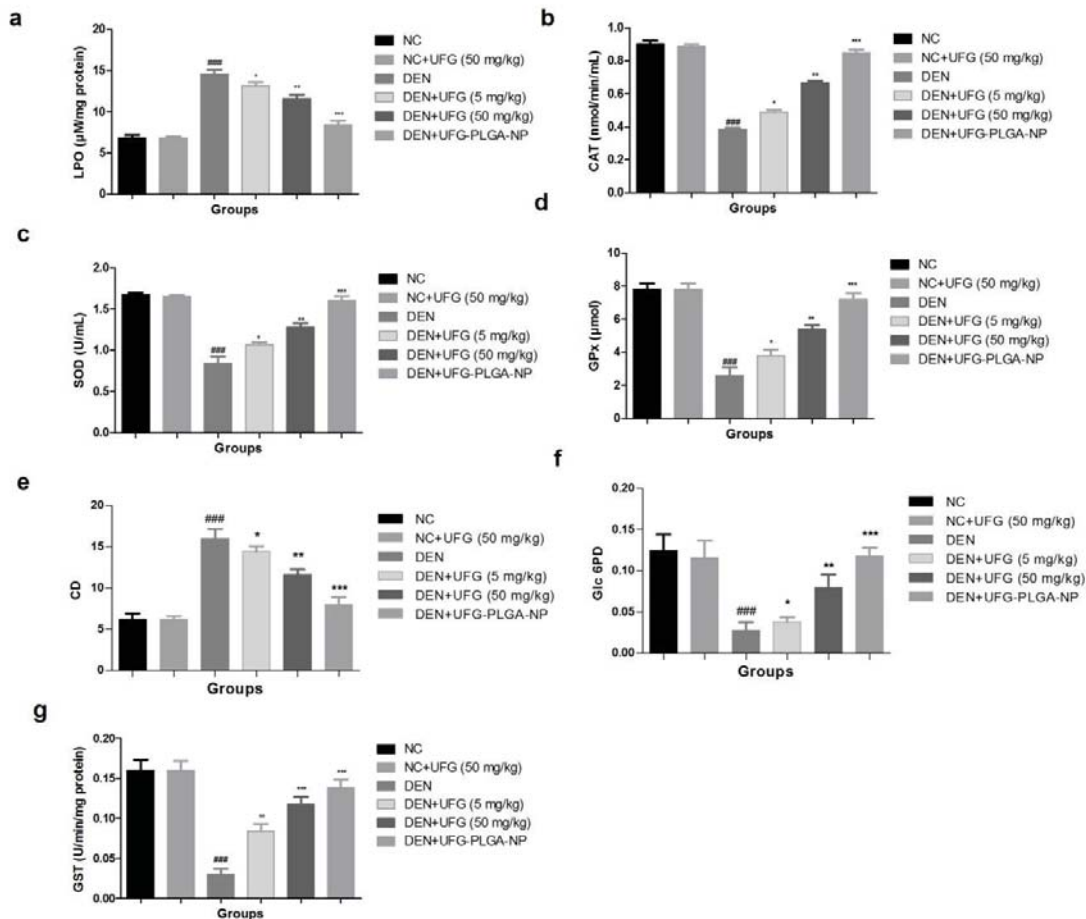
Supplementary figure 5: Effect of Umbelliferone β-D-galactopyranoside Loaded PLGA Nanoparticles (UFG-PLGA-NP) on liver parameter of DEN induced hepatocarcinogenesis in rat. Liver parameter was assessed in terms of enhanced level of (A), AFP (B) ALP (C) ALT and (D) AST as described in Materials and methods. ns = non-significant, NC= Normal control, DEN = Diethylinitrosamine, AFP= Alpha Feto Protein, AST= Aspartate Aminotransferase, ALT= Alanine transaminase, ALP= Alkaline phosphatase. DEN control group compared with normal control group ### $p < 0.001$ is consider as extremely significant and treated groups compared with the DEN control *$p < 0.05$ is considered as significant, ** $p < 0.01$ is considered as very significant, ***$p < 0.001$ is considered as extremely significant by using the ANOVA via following the Dunnett's test.
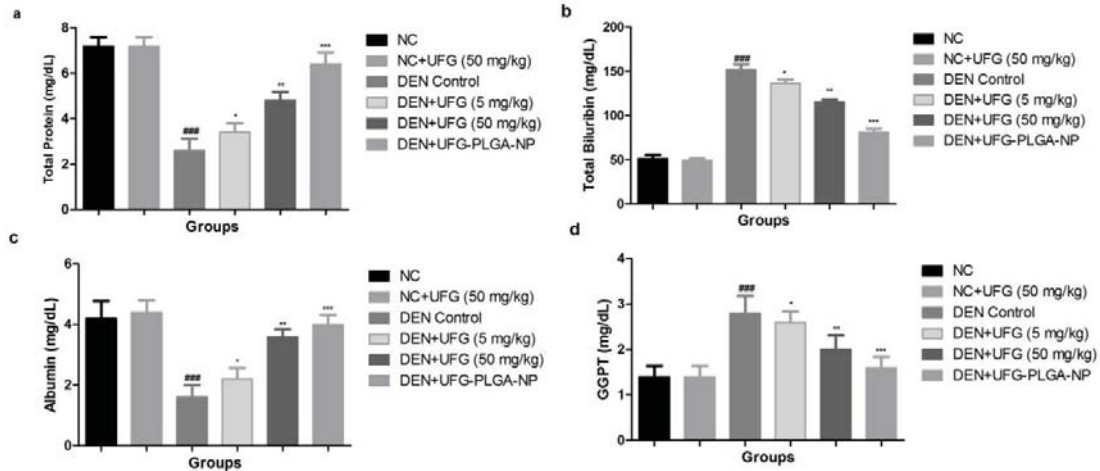
Supplementary figure 6: Effect of Umbelliferone β-D-galactopyranoside Loaded PLGA Nanoparticles (UFG-PLGA-NP) on non-liver parameter of DEN induced hepatocarcinogenesis in rat. Non-liver parameter was assessed in terms of modulated level of (A) Total protein, (B) Total Bilirubin (C) Albumin and (D) GGPT as described in materials and methods. NC= Normal control, DEN = Diethylinitrosamine, GGPT= Gamma-glutamyl transpeptidase. DEN control group compared with normal control group ### $p < 0.001$ is consider as extremely significant and treated groups compared with the DEN control *$p < 0.05$ is considered as significant, ** $p < 0.01$ is considered as very significant, ***$p < 0.001$ is considered as extremely significant by using the ANOVA via following the Dunnett's test.
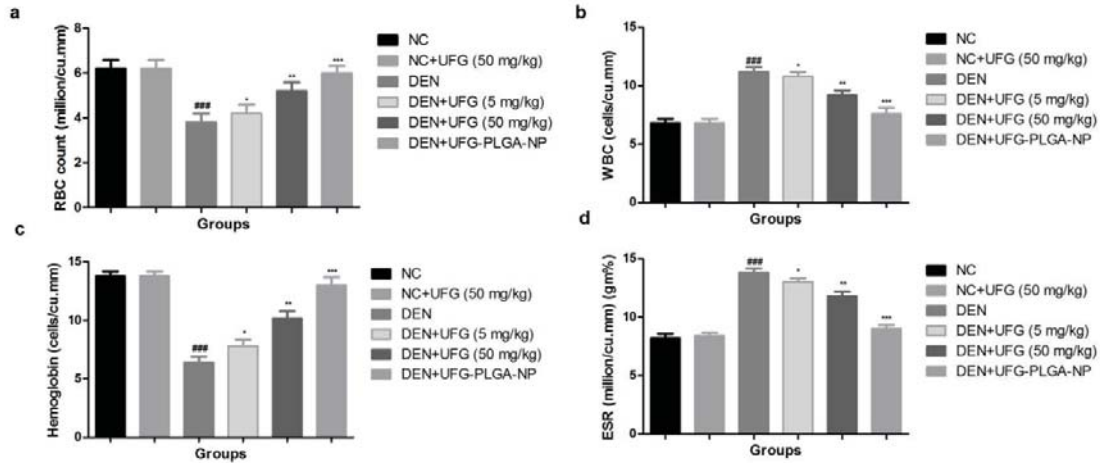
Supplementary figure 7: Effect of Umbelliferone β-D-galactopyranoside Loaded PLGA Nanoparticles (UFG-PLGA-NP) on hematological parameter of DEN induced hepatocarcinogenesis in rat. Hematological parameters were assessed in terms of altered level of (A) RBC, (B) WBC, (C) Hb and (D) ESR as described in materials and methods. NC= Normal control, DEN = Diethylinitrosamine, RBC= Red blood cell, WBC=White blood cell, Hb= Hemoglobin and ESR= Erythrocyte sedimentation rate. DEN control group compared with normal control group ### $p < 0.001$ is consider as extremely significant and treated groups compared with the DEN control *$p < 0.05$ is considered as significant, ** $p < 0.01$ is considered as very significant, ***$p < 0.001$ is considered as extremely significant by using the ANOVA via following the Dunnett's test.

Supplementary figure 8: Effect of Umbelliferone β-D-galactopyranoside Loaded PLGA Nanoparticles (UFG-PLGA-NP) on antioxidant parameters of DEN induced hepatocarcinogenesis rat. Antioxidant parameters were assessed in terms of altered level of (A) LPO, (B) CAT, (C) SOD, (D) GPx, (E) CD, (F) Glc6PD and (G) GST as described in materials and methods. NC= Normal control, DEN = Diethylinitrosamine, CAT= Catalase, SOD= Superoxide dismutase, GPx= Glutathione peroxidase, GST= glutathione transferase, Glc 6PD=Glucose 6-phosphate Dehydrogensae and CD=Conjugated dienes. DEN control group compared with normal control group ### $p < 0.001$ is consider as extremely significant and treated groups compared with the DEN control *$p < 0.05$ is considered as significant, ** $p < 0.01$ is considered as very significant, ***$p < 0.001$ is considered as extremely significant by using the ANOVA via following the Dunnett's test.