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Supporting information

for

Systematic Acute and Subchronic Toxicity Evaluation of Polysaccharide-Protein Complexes-Functionalized Selenium Nanoparticles with Anticancer Potency

Zehang Zhang [a†], Yanxin Du,[b†] Ting Liu[a], Ka-Hing Wong [c*], Tianfeng Chen [a*]

[a] The First Affiliated Hospital and Department of Chemistry, Jinan University, Guangzhou 510632, China. E-mail: tchentf@jnu.edu.cn.
[b] Orthopedics Department, Guangdong Provincial Hospital of Traditional Chinese Medicine, No. 111 Dade Road, Guangzhou 510120, China.
[c] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China
† These authors contributed equally to this work.

* To whom correspondence should be addressed. Department of Chemistry, Jinan University, Guangzhou 510632, China.
E-mail: tchentf@jnu.edu.cn.

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Results

Table S1. Summary of IC_{50} value and maximum growth inhibition (%) of different human breast cancer cells pre-treated with PTR-SeNPs for 72 h.

Cell Lines	HCC1937	MDA-MB-231	HCC1395	MCF7	HCC38	HCC1143	MCF-10A
IC_{50}(μM)	3.2	20.7	2.5	15	71	>100	79
Max Inhibition (%)	68.7	83	54.5	67	55	23	70

Table S2. Particles size, polydispersity index (PDI), zeta potential as well as *in vitro* anti-tumor activity (IC_{50}) of PTR-SeNPs during accelerated stability study.

	Month				
	0	1	2	3	6
Particle Size (nm)	80 ± 27	81.2 ± 13	81.5 ± 11	82.1 ± 18	82.5 ± 23
PDI	0.150 ± 0.02	0.154 ± 0.01	0.151 ± 0.02	0.153 ± 0.01	0.157 ± 0.02
Zeta Potential (mV)	-21.8 ± 5.62	-22.2 ± 3.37	-23.8 ± 2.23	-22.7 ± 2.81	-22.9 ± 3.09
IC_{50} (μM)	3.2	3.8	3.8	3.6	3.5

Table S3. The oral acute toxicity of PTR-SeNPs in mice and rats.

	Dose	Number		Mortality		LD_{50} (g/kg BW)	
	(g/kg BW)	Female	Male	Female	Male	Female	Male
mice	46.4	5	5	5	5	17.1	20.0
	21.5	5	5	4	3		
	10	5	5	0	0		
rat	4.64	5	5	0	0	>10	
	10	10	10	0	0		

Table S4. The mid-term hematology indicators in rats after treatment with PTR-SeNPs.

	Male (group)				Female (group)			
Parameters	Control (n=10)	Low (n=10)	Mid (n=10)	High (n=10)	Control (n=10)	Low (n=10)	Mid (n=10)	High (n=10)
RBC (10^{12}/L)	8.76 ± 0.58	8.28 ± 0.46	8.43 ± 0.25	8.56 ± 0.43	8.05 ± 0.59	8.00 ± 0.32	7.90 ± 0.47	7..77 ± 0.79
HGB (g/L)	158.3 ± 10.4	152.3 ± 7.32	153.8 ± 2.97	155.3 ± 6.13	147 ± 8.58	148.3 ± 4.83	145.7 ± 7.27	139.6 ± 11.14
WBC (10^9/L)	14.12 ± 3.66	14.05 ± 2.65	13.53 ± 1	15.04 ± 3.45	10.52 ± 2.35	10.8 ± 2.14	11.5 ± 3.55	14.39 ± 5.11
PLT (10^9/L)	699.6 ± 213.29	786.6 ± 132.79	785.5 ± 74.57	847 ± 134.06	829.3 ± 63	828.1 ± 77.42	843.1 ± 83.36	797.4 ± 157.7
HCT (%)	47.43 ± 2.52	45.85 ± 1.81	46.39 ± 1.15	47.02 ± 1.76	44.03 ± 2.42	44.31 ± 1.02	43.11 ± 2.03	42.16 ± 2.35
MCV (fL)	54.2 ± 2.14	55.43 ± 1.69	55.07 ± 1.8	55.04 ± 2.52	54.79 ± 1.54	55.45 ± 1.68	54.62 ± 1.73	54.51 ± 3.29
MCH (Pg)	18.08 ± 0.55	18.39 ± 0.49	18.26 ± 0.5	18.18 ± 0.75	18.3 ± 0.51	18.56 ± 0.67	18.46 ± 0.49	18 ± 0.67
MCHC (g/L)	333.5 ± 6.64	332.1 ± 5.3	331.5 ± 4.65	330.4 ± 3.44	333.9 ± 4.07	334.5 ± 5.02	337.8 ± 5.9	330.7 ± 11
LYM (%)	75.34 ± 5.39	78.34 ± 2.89	80.52 ± 2.75*	76.51 ± 4.2	82.1 ± 4.16	82.65 ± 6.81	82.35 ± 4.26	80.57 ± 5.92
GRAN (%)	16.61 ± 4.45	14.19 ± 2.81	12.5 ± 2.35	15.96 ± 3.51	11.57 ± 4.8	11.16 ± 6.13	10.21 ± 2.75	12.71 ± 5.82
MONO (%)	4.71 ± 1.65	5.67 ± 1.45	5.21 ± 1.39	5.2 ± 1.12	4.33 ± 1.37	4.41 ± 0.84	5.66 ± 1.49	4.74 ± 1.19
EO (%)	2.91 ± 1.73	1.57 ± 0.95	1.54 ± 0.53	2.00 ± 0.63	1.81 ± 0.57	1.6 ± 0.82	1.56 ± 0.78	1.77 ± 0.87
BASO (%)	0.43 ± 0.43	0.23 ± 0.08	0.23 ± 0.13	0.33 ± 0.17	0.19 ± 0.09	0.18 ± 0.12	0.22 ± 0.15	0.21 ± 0.1

* Significantly different from the control at $P < 0.05$.

Table S5. The mid-term urinalysis in female rats after treatment with PTR-SeNPs.

Project	Degree	Female (group)				
		Control (n=10)	Low (n=10)	Mid (n=10)	High (n=10)	P>0.05
BIL	-	10	10	10	10	
	+	0	0	0	0	
GLU	-	10	9	10	10	
	+	0	0	0	0	
KET	-	4	4	0	3	
	±	5	5	7	4	
UBG	+	1	1	3	3	
	-	10	10	10	10	
LEU	±	0	0	0	0	
	-	1	0	0	0	
	±	4	10	5	2	
	+	5	0	5	7	
	++	0	0	0	1	
PRO	-	1	3	1	2	
	±	4	6	6	4	
	+	3	1	3	3	
	++	1	0	0	1	
	+++	1	0	0	0	
BLD	-	10	10	10	10	
	±	0	0	0	0	
pH	5	0	1	0	0	
	6	3	0	0	0	
	7	8	4	7	5	
	8	2	5	3	5	
Proportion	x ± s					
			1.025 ± 0.006			
	F	1.025 ± 0.013	0.752	1.031 ± 0.010	1.031 ± 0.013	
	P		>0.05			
Color	Dark yellow			0	1	
	Light yellow			10	9	

"-" represents negative; "+" represents positive; additonal "+" represents deeper.

Table S6. The mid-term urinalysis in male rats after treatment with PTR-SeNPs.

Project	Degree	Male (group)				
		Control (n=10)	Low (n=10)	Mid (n=10)	High (n=10)	P>0.05
BIL	-	10	10	10	10	
	+	0	0	0	0	
GLU	-	10	9	10	10	
	+	0	1	0	0	
KET	-	10	10	10	10	
	±	0	0	0	0	
UBG	-	10	10	10	10	
	±	0	0	0	0	
LEU	-	10	10	10	10	
	±	0	0	0	0	
PRO	-	2	4	2	2	
	±	6	3	5	7	
	+	0	3	3	1	
	++	2	0	0	0	
BLD	-	10	10	10	10	
	±	0	0	0	0	
pH	5	2	3	2	3	
	6	0	0	0	0	
	7	2	1	2	4	
	8	6	6	6	3	
proportion	x ± s		1.023 ± 0.007			
	F	1.029 ± 0.009	0.694	1.026 ± 0.009	1.021 ± 0.007	
	P		>0.05			
Color	Light red	0	0	1	0	
	Light green	0	0	0	1	
	Dark yellow	0	1	0	2	
	Light yellow	10	9	9	7	

"-" represents negative; "+" represents positive; additional "+" represents deeper.

Table S7. The end-term urinalysis in male rats after treatment with PTR-SeNPs.

Project	Degree	Male (group)				
		Control (n=10)	Low (n=10)	Mid (n=10)	High (n=10)	P>0.05
BIL	-	10	10	10	10	
	+	0	0	0	0	
GLU	-	10	10	10	9	
	+	0	0	0	1	
KET	-	2	1	1	1	
	±	5	7	7	6	
	+	3	2	2	3	
UBG	-	10	10	10	10	
	±	0	0	0	0	
LEU	-	5	2	2	3	
	±	3	5	3	3	
	+	2	3	4	3	
	++	0	0	0	1	
PRO	-	3	0	0	1	
	±	1	4	1	3	
	+	2	0	1	1	
	++	3	3	5	2	
	+++	1	3	3	3	
BLD	-	10	10	10	10	
		0	0	0	0	
pH	4	0	0	0	1	
	5	0	0	0	0	
	6	3	0	0	0	
	7	0	0	1	1	
	8	3	5	2	5	
	9	4	5	7	3	
Proportion	x ± s					
			1.027 ± 0.008			
	F	1.025 ± 0.007	0.859	1.029 ± 0.005	1.030 ± 0.012	
Color	Colorless	0	0	0	1	
	Light red	0	3	2	1	
	Light yellow	10	7	8	8	

"-" represents negative; "+" represents positive; additional "+" represents deeper.

Table S8. The end-term urinalysis in female rats after treatment with PTR-SeNPs.

Project	Degree	Female (group)				
		Control (n=10)	Low (n=10)	Mid (n=10)	High (n=10)	P>0.05
BIL	-	10	10	10	10	
	+	0	0	0	0	
GLU	-	10	10	10	10	
	+	0	0	0	0	
KET	-	10	10	10	10	
	±	0	0	0	0	
UBG	-	10	9	10	10	
	±	0	1	0	0	
LEU	-	10	8	10	9	
	±	0	1	0	0	
	+	0	1	0	0	
	++	0	0	0	0	
	+++	0	0	0	1	
PRO	-	2	2	1	1	
	±	6	5	3	6	
	+	2	2	3	0	
	++	0	1	3	3	
BLD	-	6	5	7	5	
	±	2	3	3	5	
	+	2	2	0	0	
pH	4	0	0	1	0	
	5	4	3	0	1	
	6	2	2	2	2	
	7	3	1	1	4	
	8	1	3	6	3	
Proportion	x ± s		1.023 ± 0.007			
	F	1.029 ± 0.011	1.738	1.029 ± 0.01	1.025 ± 0.012	
	P		0.177			
Color	Dark red	0	1	0	0	
	Light yellow	10	6	10	9	
	Dark brown	0	1	0	0	
	Dark orange	0	2	0	1	

"-" represents negative; "+" represents positive; additional "+" represents deeper.

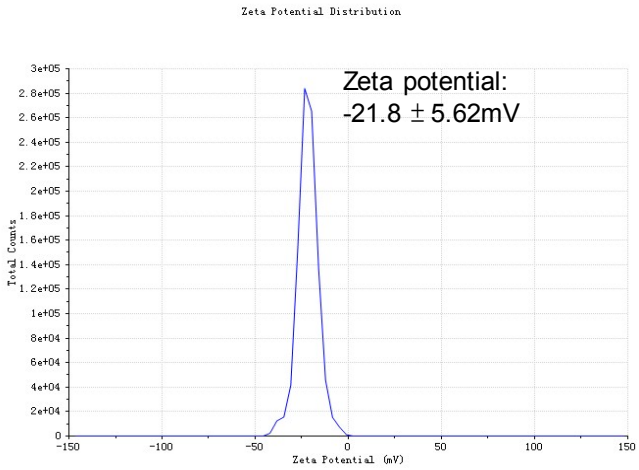

Figure S1. The zeta potential of PTR-SeNPs.

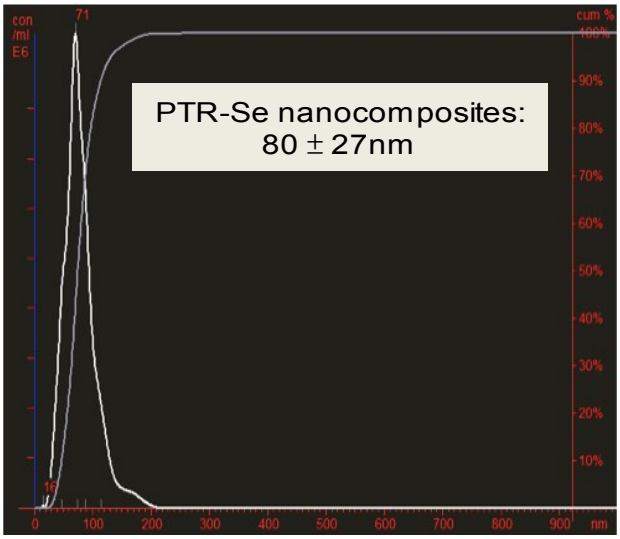

Figure S2. The size of PTR-SeNPs.

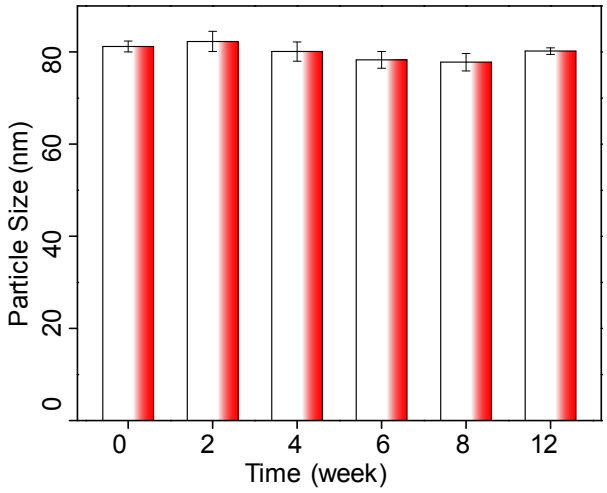

Figure S3. Stability analysis of PTR-SeNPs.

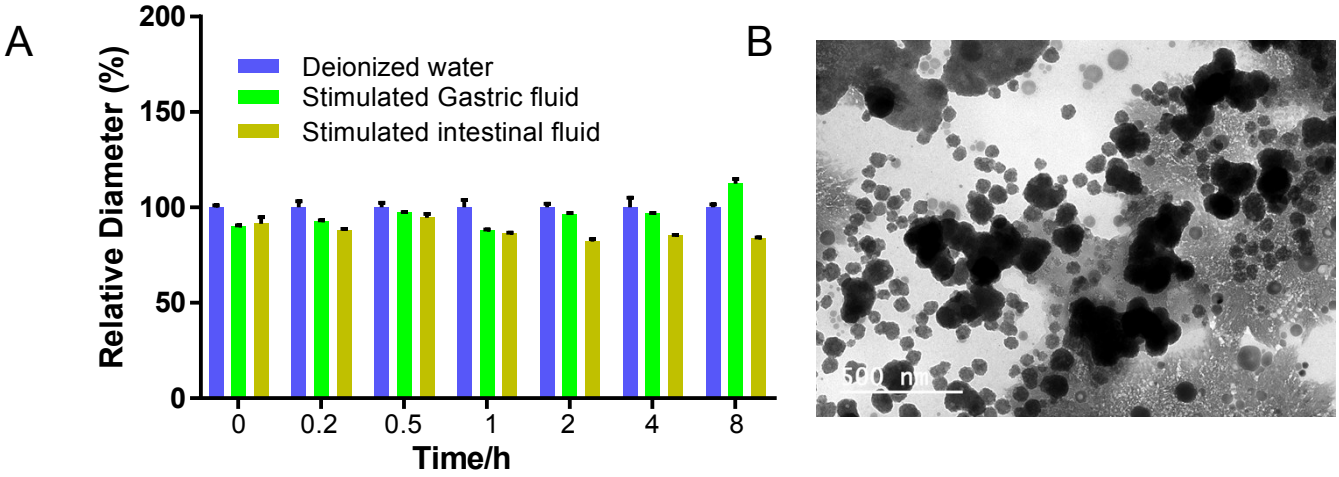

Figure S4. Stability of PTR-SeNPs in stimulated gastric and intestinal fluid. (A) Relative diameter (%) change of PTR-SeNPs in simulated gastric and intestinal fluid. (B) TEM image of PTR-SeNPs under the simulated intestinal fluid for 8 h.

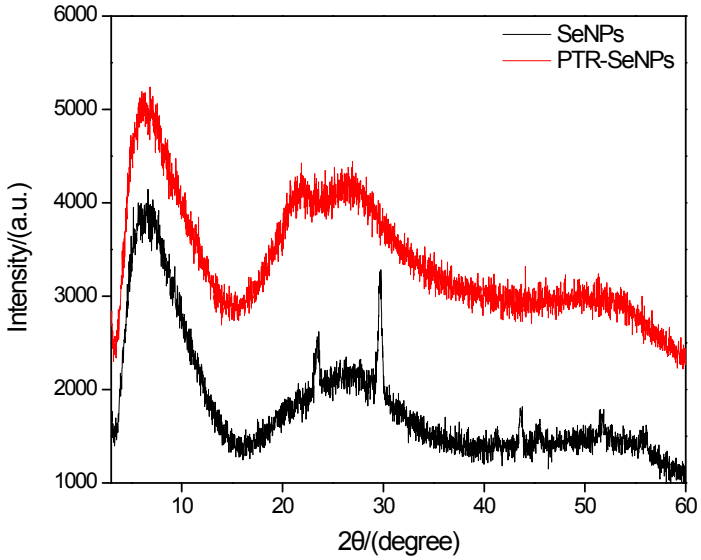

Figure S5. XRD spectra of PTR-SeNPs and SeNPs.

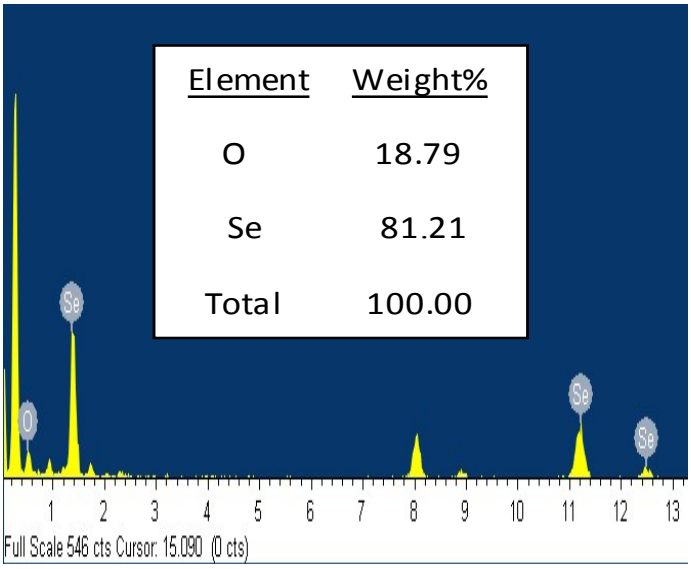

Figure S6. EDX of PTR-SeNPs.

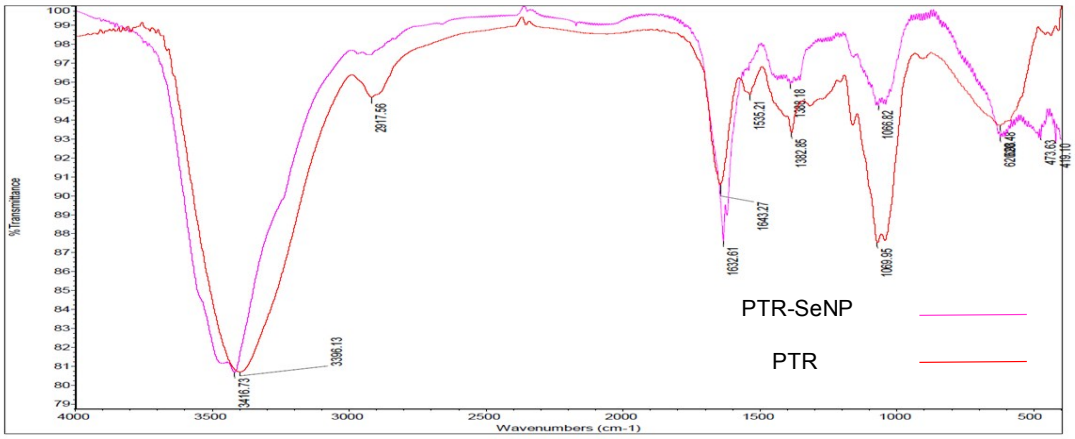

Figure S7. FT-IR of PTR and PTR-SeNPs.

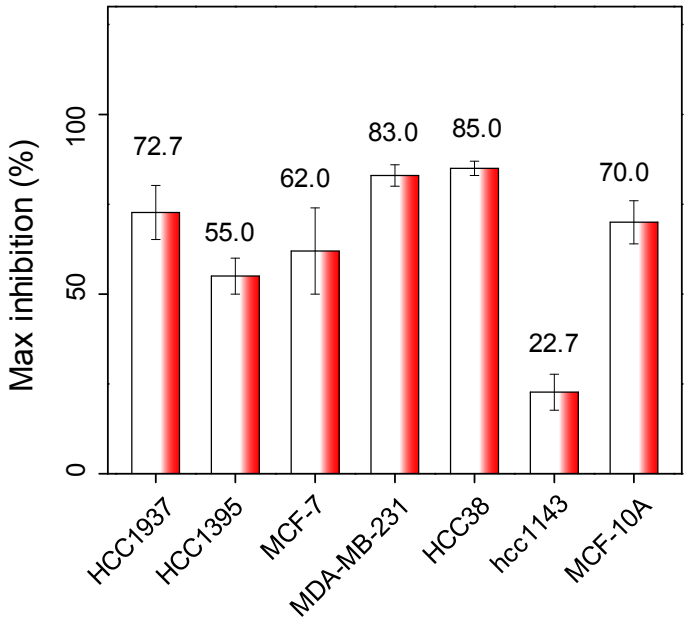

Figure S8. Maximum growth inhibition rate (%) of different human breast cancer cells treated with PTR-SeNPs for 72 h. Both MTS and BrdU assays determined cell viability.

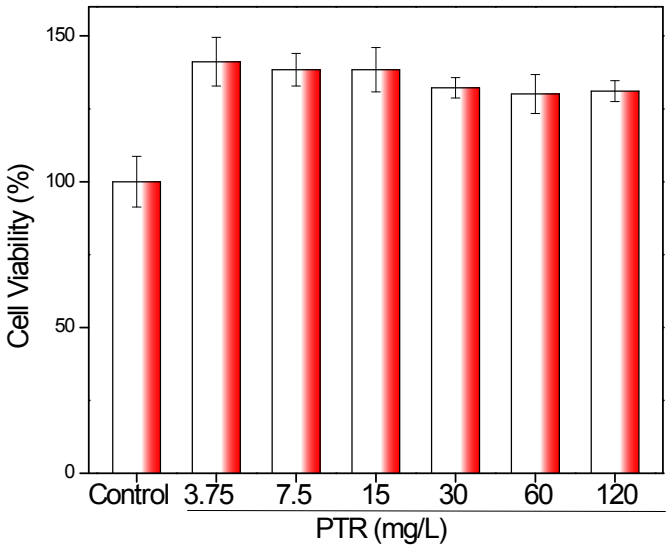

Figure S9. The cell viability of HCC1937 cells treated with different concentration of PTR (3.75 mg/L ~ 120 mg/L) for 72 h.

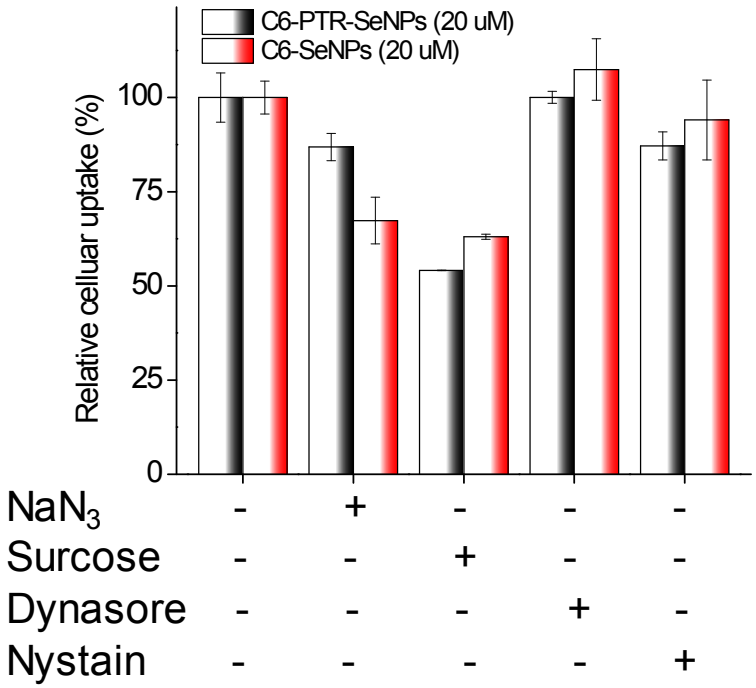

Figure S10. Cellular uptake of HCC1937 cells are pre-treated with inhibitors (NaN_3, sucrose, dynastore and nystatin) for 1 h before adding 20 μM of C6-PTR-SeNPs and incubates at 37 ℃ for 3 h

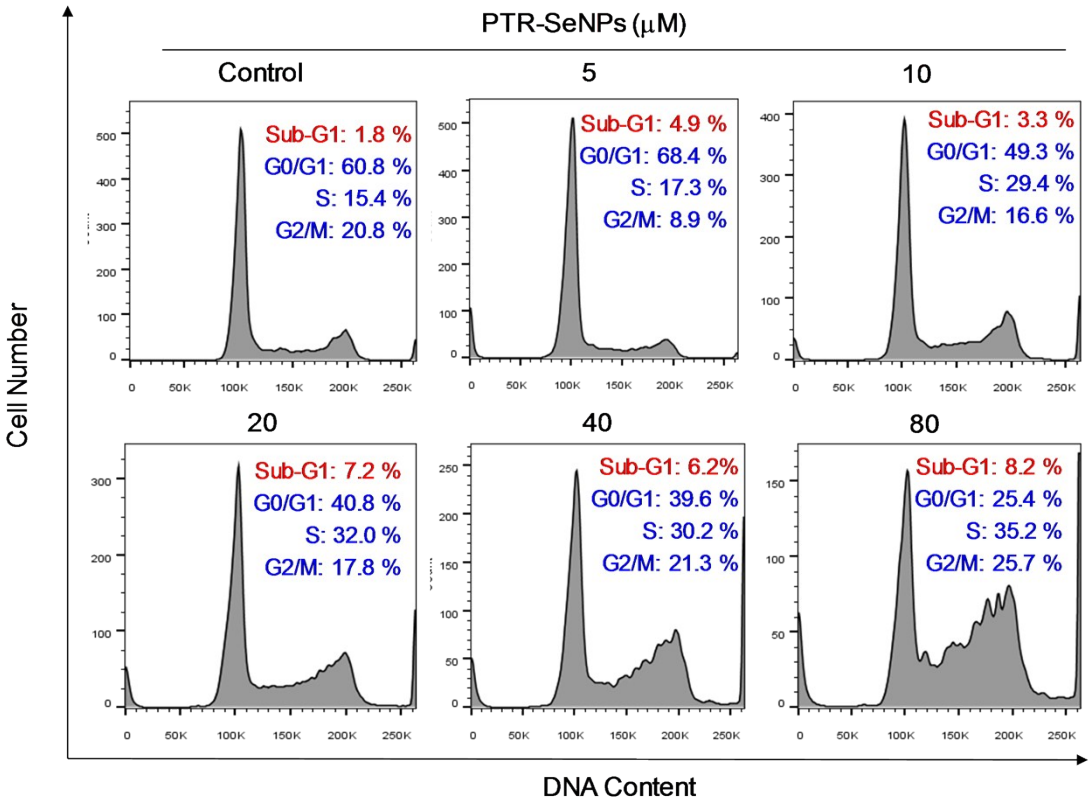

Figure S11. Flow cytometric analysis of cell cycle distribution and apoptosis in MDA-MB-231 cells pretreated with different concentrations of PTR-SeNPs (5-80 μM) for 72 h. After PI staining, cellular DNA histograms were analyzed by the MultiCycle software. The apoptotic cell death was quantified by measuring the sub-G1 cell population.

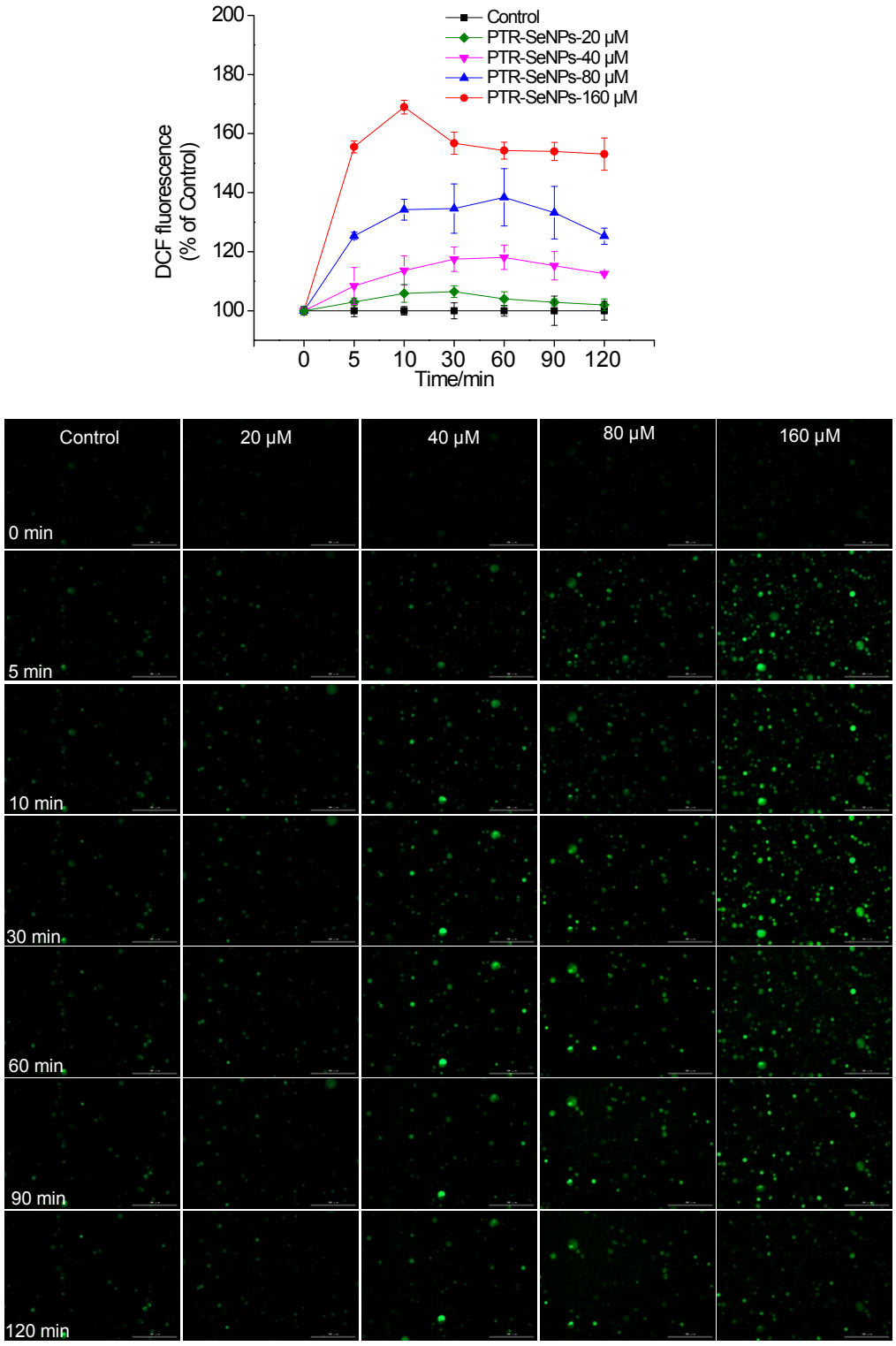

Figure S12. Intracellular ROS generation in HCC1937 cells induced by PTR-SeNPs. Cells were treated with different concentrations of C6-PTR-SeNPs (20 μM ~ 160 μM) and immediately tested the level of ROS. Intracellular levels of ROS in treated cells is expressed as percentage of control cells. ROS generation was determined by measuring the fluorescence intensity of an oxidation-sensitive fluorescence DCFH-DA.

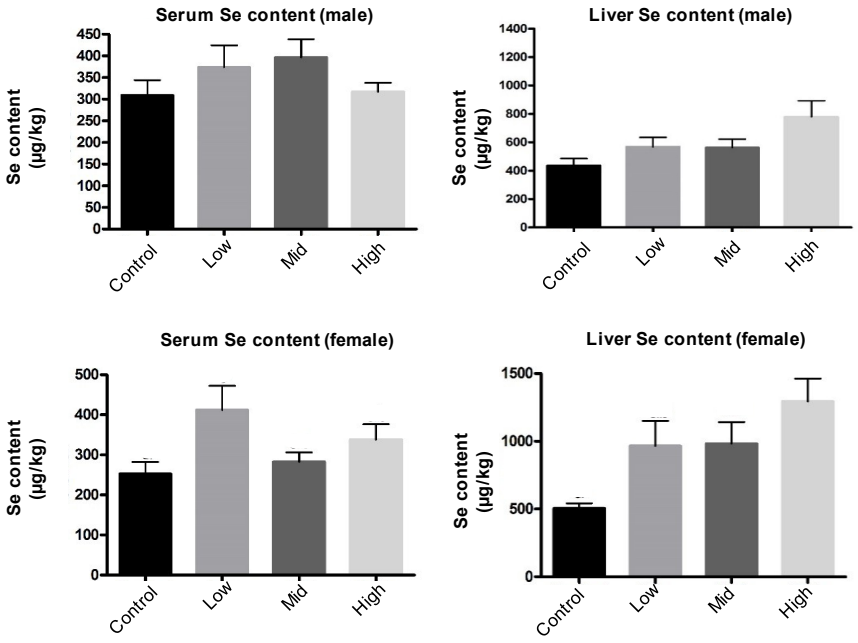

Figure S13. Effect of PTR-SeNPs in liver and serum Se content in rats after treatment with 0.017 (Low), 0.055 (Mid) and 0.167 (High) g/kg BW/day PTR-SeNPs for 90 days.

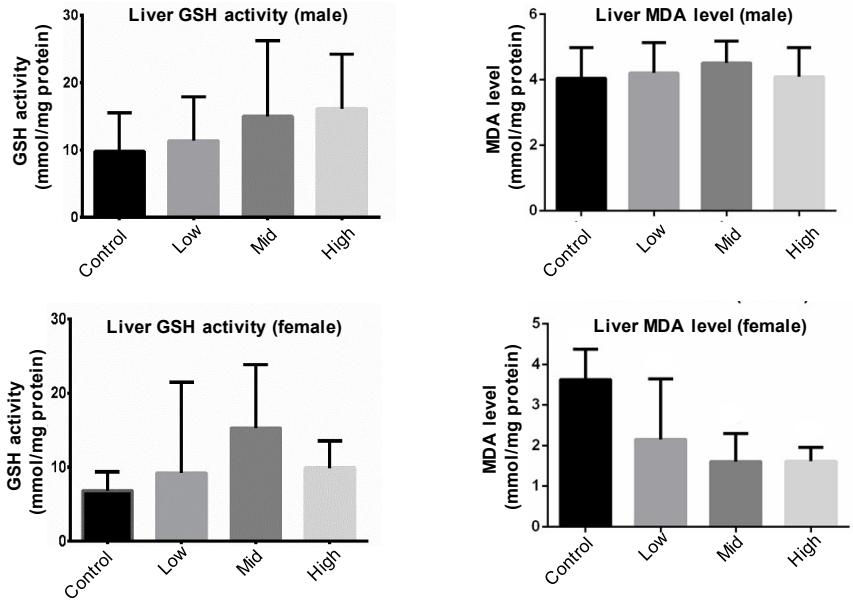

Figure S14. Effect of PTR-SeNPs in liver on liver oxidative stress in rats after treatment with 0.017 (Low), 0.055 (Mid) and 0.167 (High) g/kg BW/day PTR-SeNPs for 90 days.