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Heparin-coated iron oxide nanoparticles: application as a liver contrast agent, toxicity and pharmacokinetics

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Supporting information

Table S1. List of bacterial strains and controls used in the Ames test

Strain	Mutations	Plasmid	S9 supplementation	Positive chemical control	Control concentration (μg/plate)
Salmonella typhimurium TA100	*hisG46 uvrBrfa*	pKM101	Yes	Benzo(a)pyrene	5
			No	Sodium azide	5
Salmonella typhimurium TA1535	*hisG46 uvrBrfa*	–	Yes	2-aminoanthracene	2
			No	Sodium azide	5
Escherichia coli WP2 uvr2	*trpEuvrA*	–	Yes	2-aminoanthracene	10
			No	4-nitroquinoline-N-oxide	0.5

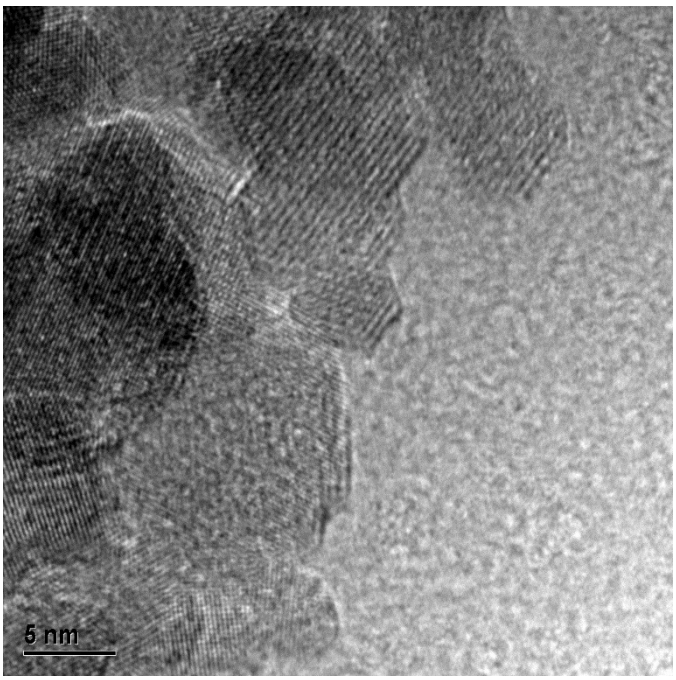

Figure S1. TEM images of IONPs

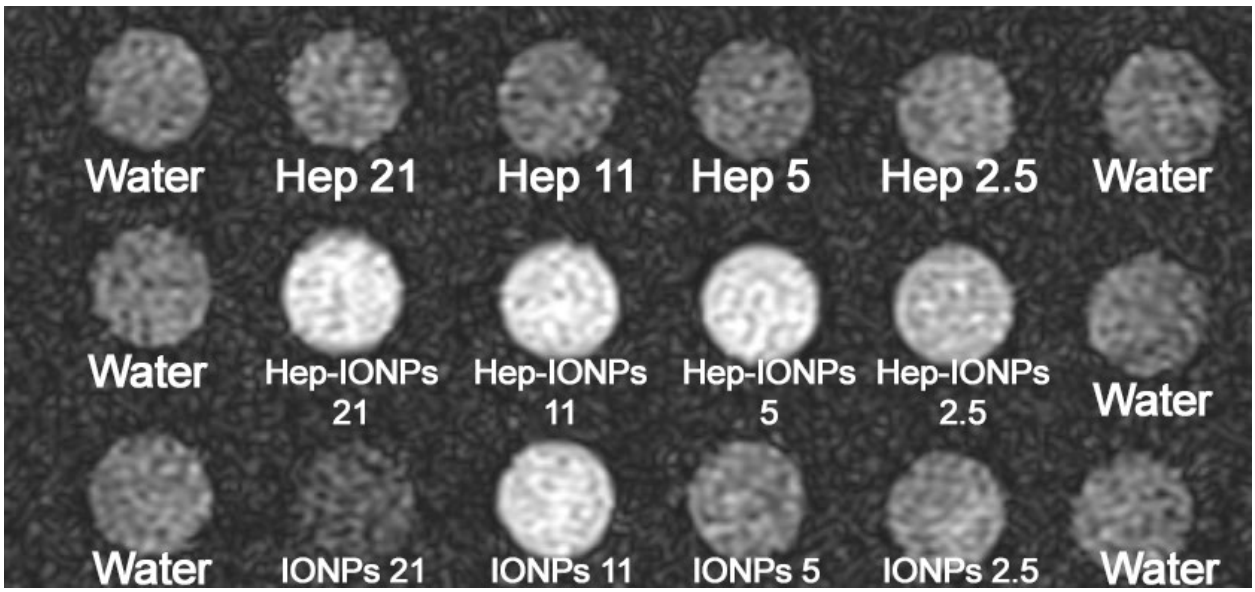

Figure S2. MRI scan of different concentrations of NPs. Concentration given in mmol/L

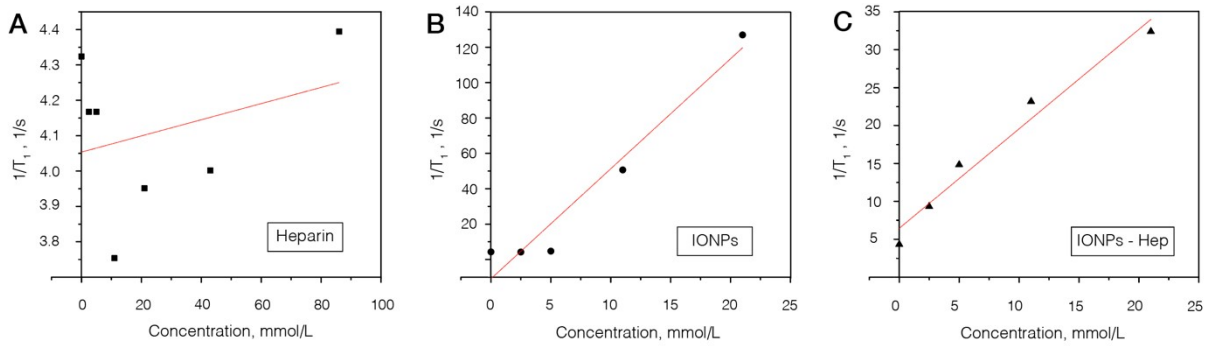

Figure S3. Dependence of $1/T_1$ from concentrations of nanoparticles

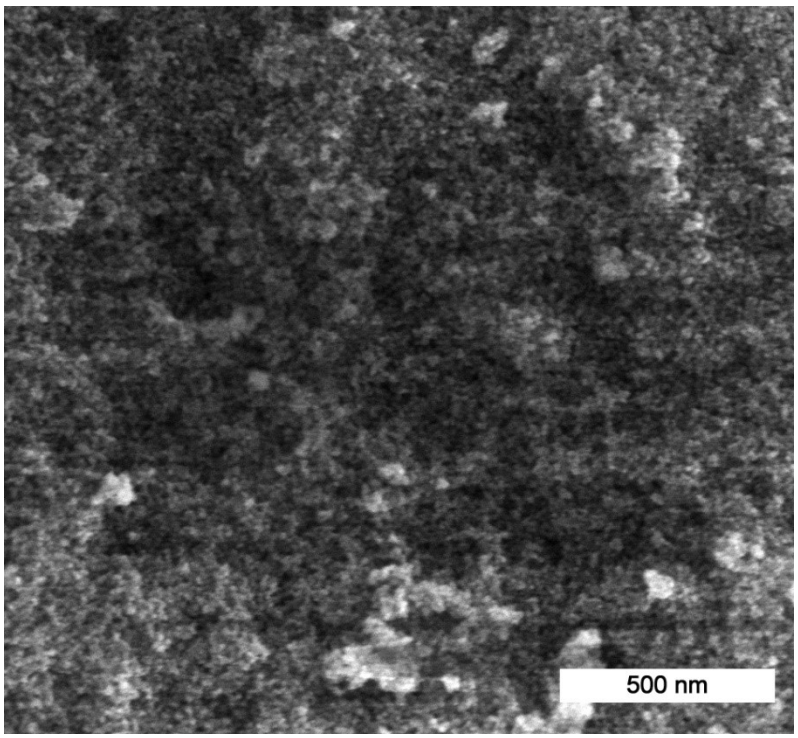

Figure S4. SEM images of Hep-IONPs

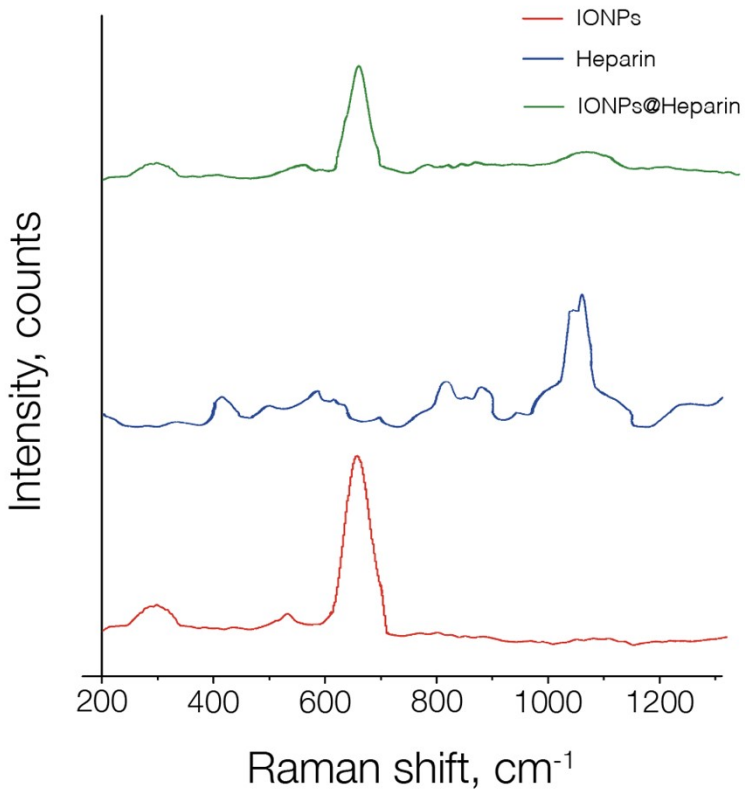

Figure S5. Raman spectra of IONPs, heparin and Hep-IONPs. At 30 μW, magnetite can be identified by 678 cm-1 band

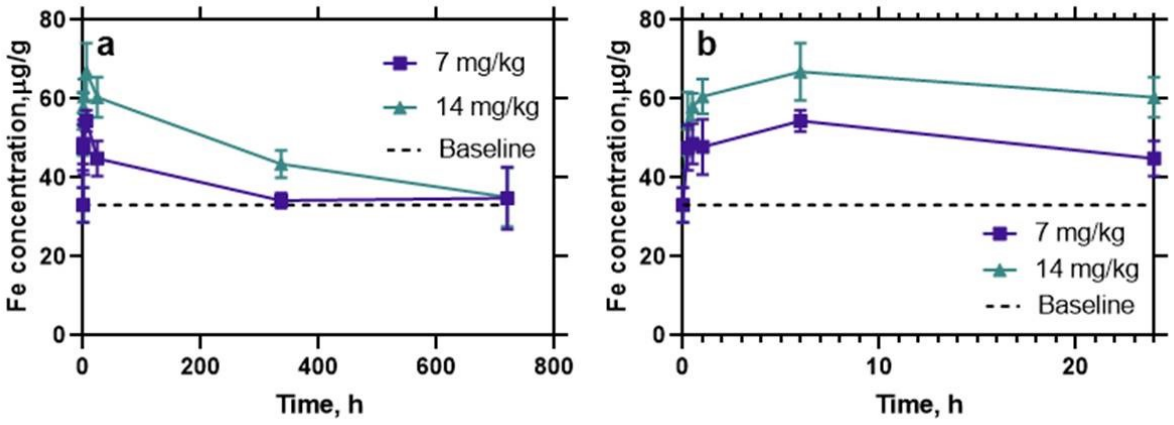

Figure S6. Pharmacokinetic curves of total iron in rat liver tissue at doses of 7 mg/kg **and 10 mg/kg: a) full curve, b) initial section (0–24h)**

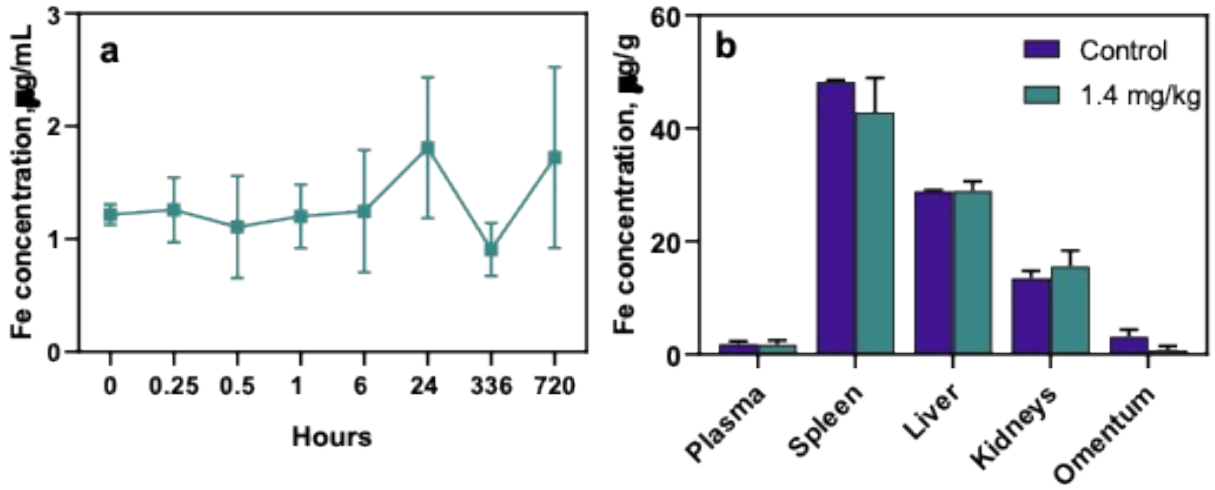

Figure S7. (a) Pharmacokinetic curve of total iron in rabbits' plasma at dose 1.4 mg/kg. (b) total iron level in plasma and internal organs of rabbits at dose of 1.4 mg/kg

Table S2. Influence of daily i.v. administrations of Hep-IONPs on the rats' food consumption

Time point	Experimental group and gender							
	Control		1.4 mg/kg		7 mg/kg		14 mg/kg	
	Males	Females	Males	Females	Males	Females	Males	Females
Baseline	21.2	16.8	18.8	17.7	18.4	17.8	20.0	16.8
8 days	23.3	17.1	19.1	18.0	19.9	18.6	22.0	17.6
15 days	18.4	13.2	17.3	16.0	18.6	16.7	19.1	16.7
23 days	24.3	19.5	27.3	20.8	23.0	24.2	30.0	21.2
30 days	22.2	20.3	25.2	20.8	23.8	20.8	25.8	19.7
37 days	29.7	17.8	23.7	19.3	20.5	16.7	26.2	18.8
44 days	24.2	17.5	23.7	17.2	20.5	16.5	23.3	16.8

Table S3. Influence of daily i.v. administrations of Hep-IONPs on the rats' water consumption

Time	Experimental group and gender

point	Control		1.4 mg/kg		7 mg/kg		14 mg/kg	
	Males	Females	Males	Females	Males	Females	Males	Females
Baseline	34.2	27.5	29.2	30.8	33.3	30.8	35.8	30.8
8 days	35.0	24.2	31.3	30.8	31.7	27.5	33.3	26.7
15 days	34.8	21.3	35.7	26.6	35.2	31.6	32.0	31.4
23 days	41.7	30.0	48.3	38.3	38.3	36.7	45.0	33.3
30 days	33.3	33.3	41.7	40.0	37.5	37.5	50.0	37.5
37 days	45.8	29.2	41.7	33.3	33.3	33.3	43.3	37.5
44 days	35.0	25.0	35.0	29.2	33.3	30.0	40.0	29.2

Table S4. Influence of daily i.v. administrations of Hep-IONPs on the frequency of respiratory movements of rats (respiratory movements / min, M ± SD)

Time point	Experimental group and gender			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Males				
Baseline	131.1±25.2	144.3±32.0	132.1±22.9	147.3±29.7
14 days	133.9±16.7	132.4±21.5	124.1±17.3	138.6±23.2
30 days	122.5±16.4	124.2±11.0	119.2±13.8	121.7±18.0
Females				
Baseline	151.3±29.8	143.3±20.3	143.3±20.3	144.3±22.4
14 days	127.4±17.8	129.8±20.8	129.8±20.8	133.8±19.9
30 days	118.5±10.5	124.2±13.7	118.8±15.8	115.0±12.9

Table S5. Influence of daily i.v. administrations of Hep-IONPs on the electrocardiogram parameters of male rats (M ± SD)

Parameter	Experimental group and gender			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Baseline values, males				
P, mV	0.11±0.04	0.12±0.05	0.12±0.03	0.12±0.05
R, mV	0.29±0.10	0.35±0.07	0.33±0.12	0.24±0.07
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.23±0.07	0.21±0.08	0.21±0.12	0.21±0.08
PQ, ms	42.2±5.5	43.2±4.0	41.5±6.6	42.0±4.9
QT, ms	69.7±10.7	68.1±6.3	67.5±7.5	75.5±9.8
Heart rate	513.8±28.5	516.6±30.7	499.8±23.4	508.4±35.7
14 days, males				
P, mV	0.12±0.05	0.13±0.03	0.14±0.07	0.15±0.05
R, mV	0.31±0.09	0.34±0.09	0.37±0.09	0.27±0.11
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.18±0.08	0.20±0.07	0.19±0.10	0.25±0.10
PQ, ms	43.4±5.0	46.6±3.4	47.7±7.0	44.6±3.3
QT, ms	64.8±8.5	66.0±5.6	65.8±6.3	68.3±9.6
Heart rate	509.8±49.8	507.7±21.8	496.4±27.8	515.2±31.4

Table S6. Influence of daily i.v. administrations of Hep-IONPs on the electrocardiogram parameters of female rats (M ± SD)

Parameter	Parameter			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Baseline values, females				
P, mV	0.12±0.04	0.10±0.02	0.13±0.05	0.11±0.04
R, mV	0.25±0.05	0.30±0.06	0.32±0.12	0.25±0.07
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.23±0.08	0.24±0.08	0.20±0.06	0.17±0.05
PQ, ms	42.6±4.2	41.0±7.1	42.3±2.6	41.1±2.2
QT, ms	66.3±5.1	68.2±8.5	65.3±5.9	63.2±4.2
Heart rate	513.9±33.9	525.3±40.8	530.3±33.4	545.8±27.5
14 days, females				
P, mV	0.15±0.07	0.13±0.03	0.16±0.07	0.15±0.02
R, mV	0.29±0.08	0.31±0.08	0.40±0.12	0.34±0.08
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.23±0.07	0.24±0.06	0.24±0.08	0.27±0.10
PQ, ms	46.7±6.5	44.8±4.5	46.2±4.6	44.5±4.1
QT, ms	62.8±4.9	65.9±5.5	66.9±6.3	67.7±5.6
Heart rate	509.3±28.9	505.5±44.0	528.2±32.2	525.4±26.7

Table S7. Influence of daily i.v. administrations of Hep-IONPs on the rabbits' food consumption

Time point	Experimental group and gender							
	Control		1.4 mg/kg		7 mg/kg		14 mg/kg	
	Males	Females	Males	Females	Males	Females	Males	Females
Baseline	77.8	72.5	96.7	89.5	100.8	131.5	97.0	103.3
8 days	120.7	127.5	122.5	108.2	146.0	141.8	142.5	113.0
15 days	144.0	137.5	138.7	134.7	128.7	133.8	118.0	126.5
23 days	152.3	184.3	170.0	175.7	174.0	180.3	203.3	162.0
30 days	152.7	173.3	167.3	154.3	134.7	169.3	196.3	167.0
37 days	151.7	180.0	168.7	171.0	141.7	154.7	185.7	159.3
44 days	173.7	157.3	166.0	199.7	156.3	176.7	168.0	186.7

Table S8. Influence of daily i.v. administrations of Hep-IONPs on the rabbits' water consumption

Time point	Experimental group and gender							
	Control		1.4 mg/kg		7 mg/kg		14 mg/kg	
	Males	Females	Males	Females	Males	Females	Males	Females
Baseline	203.0	106.8	201.8	145.2	214.5	256.5	225.0	167.8
8 days	216.7	166.7	173.3	160.0	283.3	231.7	215.0	228.3
15 days	278.3	283.3	275.0	281.7	300.0	318.3	205.0	235.0
23 days	216.7	360.0	250.0	400.0	346.7	376.7	356.7	293.3
30 days	265.0	416.7	430.0	423.3	446.7	300.0	368.3	270.0
37 days	246.7	263.3	316.7	370.0	333.3	300.0	326.7	368.3
44 days	316.3	283.3	457.3	478.7	484.3	266.3	285.3	321.3

Table S9. Influence of daily i.v. administrations of Hep-IONPs on the frequency of respiratory movements of rabbits (respiratory movements / min, M ± SD)

Time point	Experimental group and gender			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Males				
Baseline	118.5±13.8	103.0±14.0	108.0±22.5	114.0±11.9
14 days	108.5±16.7	115.0±10.0	130.5±27.9	119.0±10.2
30 days	153.0±7.9	159.0±54.2	150.0±31.3	134.0±45.9
Females				
Baseline	103.0±7.8	119.0±9.2	111.5±15.1	123.0±15.5
14 days	121.0±20.0	130.5±14.5	120.0±27.8	118.0±13.2
30 days	126.7±28.0	164.0±40.8	164.0±16.5	143.0±31.5

Table S10. Influence of daily i.v. administrations of Hep-IONPs on the electrocardiogram parameters of male rabbits (M ± SD)

Parameter	Experimental group and gender			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Baseline values, males				
P, mV	0.18±0.04	0.20±0.05	0.18±0.03	0.16±0.02
R, mV	0.31±0.10	0.39±0.20	0.42±0.12	0.34±0.10
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.17±0.08	0.17±0.09	0.23±0.09	0.12±0.06
PQ, ms	60.0±7.1	59.0±4.7	62.2±8.6	63.7±7.4
QT, ms	134.3±15.3	134.0±17.1	135.8±15.7	138.8±14.2
Heart rate	290.3±62.3	287.8±47.9	238.8±55.4	255.2±39.0
14 days, males				
P, mV	0.17±0.02	0.17±0.06	0.17±0.03	0.15±0.02
R, mV	0.27±0.12	0.26±0.09	0.31±0.10	0.33±0.10
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.17±0.05	0.12±0.02	0.18±0.06	0.14±0.08
PQ, ms	57.3±4.1	61.2±5.6	58.7±8.5	66.7±4.8
QT, ms	118.8±13.1	135.3±10.4	127.7±10.9	134.3±12.2
Heart rate	316.0±45.5	289.2±29.3	296.0±67.7	258.3±53.0

Table S11. Influence of daily i.v. administrations of Hep-IONPs on the electrocardiogram parameters of female rabbits (M ± SD)

Parameter	Parameter			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Baseline values, females				
P, mV	0.19±0.07	0.19±0.03	0.15±0.05	0.16±0.03
R, mV	0.27±0.07	0.32±0.13	0.30±0.10	0.40±0.14
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.19±0.10	0.13±0.05	0.16±0.07	0.15±0.09
PQ, ms	59.7±5.1	56.5±8.1	61.7±10.1	62.8±10.7
QT, ms	125.7±9.3	123.2±9.7	131.7±20.8	134.2±9.0
Heart rate	309.0±64.4	333.8±41.6	257.0±48.8	265.8±35.8
14 days, females				
P, mV	0.14±0.04	0.17±0.02	0.14±0.02	0.12±0.02

R, mV	0.28±0.08	0.32±0.12	0.33±0.08	0.34±0.08
S, mV	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T, mV	0.15±0.04	0.17±0.08	0.15±0.03	0.17±0.08
PQ, ms	65.2±12.0	62.3±6.7	64.7±5.8	64.5±7.6
QT, ms	127.3±7.2	131.3±5.9	138.5±21.8	131.7±7.2
Heart rate	289.2±46.3	294.2±15.0	236.2±39.4	278.0±36.4

Table S12. Influence of daily i.v. administrations of Hep-IONPs on the peripheral blood parameters of rats (M±SD), on 14th day

Parameter	Experimental groups			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
14 days, male				
Leukocyte count (WBC), 10^9/L	17.6±1.4	14.1±2.6	18.6±3.2	13.4±4.2
Lymphocytes (LYM), 10^9/L	11.5±2.5	9.6±1.7	12.3±2.2	8.4±3.4
Monocytes and eosinophils (MID), 10^9/L	1.2±0.5	0.9±0.3	1.2±0.3	0.8±0.3
Granulocytes (GRAN), 10^9/L	5.0±0.9	3.6±0.8	5.1±1.6	4.2±1.3
Erythrocyte count (RBC), 10^{12}/L	5.50±0.74	5.54±0.40	5.21±0.39	5.65±0.61
Hemoglobin concentration (HGB), g/L	83.2±13.3	88.5±10.9	84.7±10.8	92.8±13.5
Hematocrit (HCT), %	25.0±3.9	26.1±2.3	25.1±2.5	27.6±2.8
Mean erythrocyte volume (MCV), fL	45.6±1.5	47.2±1.5	48.2±2.2	49.0±1.5*
Mean corpuscular hemoglobin (MCH), pg	15.1±0.8	15.9±0.9	16.1±1.1	16.3±0.6
Mean corpuscular hemoglobin concentration (MCHC), g/dL	332.2±14.4	338.8±23.7	336.3±17.9	334.5±17.1
Thrombokrit (PCT), %	0.16±0.08	0.16±0.07	0.16±0.06	0.14±0.04
14 days, female				
Leukocyte count (WBC), 10^9/L	12.6±2.1	10.4±2.5	12.7±3.1	11.7±3.6
Lymphocytes (LYM), 10^9/L	9.2±2.0	7.0±2.9	8.2±1.5	7.8±2.4
Monocytes and eosinophils (MID), 10^9/L	0.8±0.2	0.7±0.2	1.2±0.6	0.8±0.3
Granulocytes (GRAN), 10^9/L	2.6±0.2	2.7±0.6	3.4±1.5	3.2±1.3
Erythrocyte count (RBC), 10^{12}/L	5.04±0.33	5.55±0.67	5.14±0.58	4.82±0.53
Hemoglobin concentration (HGB), g/L	79.2±9.7	98.5±16.9	87.7±14.6	79.3±10.0
Hematocrit (HCT), %	23.6±1.7	27.3±3.0	26.0±3.2	24.1±1.6
Average erythrocyte volume (MCV), fL	47.0±2.3	49.4±3.0	50.5±2.4	50.3±2.4
Mean corpuscular hemoglobin (MCH), pg	15.6±1.2	17.7±2.0	16.9±1.3	16.4±0.9
Mean corpuscular hemoglobin concentration (MCHC), g/dL	333.8±19.2	358.2±30.9	336.0±17.5	328.2±21.2
Thrombokrit (PCT), %	0.16±0.06	0.16±0.03	0.14±0.04	0.14±0.03

*- differences with the control group are statistically significant ($p < 0.05$); the Kruskal-Wallis test for independent samples was used for assessment of the significance of differences between control and experimental groups

Table S13. Influence of daily i.v. administrations of Hep-IONPs on the peripheral blood parameters of rats (M±SD), on 44th day

Parameter	Experimental groups			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
30 days after the last injection, male				
Leukocyte count (WBC), 10^9/L	15.7±6.7	17.7±7.1	23.3±4.3	16.1±7.5
Lymphocytes (LYM), 10^9/L	10.6±5.3	11.6±4.9	15.9±3.2	9.5±5.1
Monocytes and eosinophils (MID), 10^9/L	1.0±0.4	1.3±0.8	1.4±0.2	1.2±0.8
Granulocytes (GRAN), 10^9/L	4.1±1.1	4.8±1.9	6.0±1.2	5.4±2.8
Erythrocyte count (RBC), 10^{12}/L	6.52±0.55	7.03±0.50	7.36±0.53	7.34±0.36
Hemoglobin concentration (HGB), g/L	113.3±11.0	125.7±11.0	128.0±11.0	130.3±6.1
Hematocrit (HCT), %	26.0±2.8	27.6±2.3	30.1±2.1	31.2±2.1*
Average erythrocyte volume (MCV), fL	39.8±1.4	39.3±1.1	41.0±1.6	42.6±1.1
Mean corpuscular hemoglobin (MCH), pg	17.3±1.0	17.8±1.2	17.4±0.4	17.7±0.5
Mean corpuscular hemoglobin concentration (MCHC), g/dL	437.5±39.4	455.7±26.4	424.3±21.0	417.3±13.4
Thrombokrit (PCT), %	0.20±0.11	0.16±0.07	0.15±0.04	0.21±0.05
30 days after the last injection, female				
Leukocyte count (WBC), 10^9/L	11.8±4.0	14.1±2.8	17.5±4.3	19.8±5.8*
Lymphocytes (LYM), 10^9/L	8.2±3.2	9.3±2.6	12.0±2.6	13.4±4.5
Monocytes and eosinophils (MID), 10^9/L	0.8±0.3	1.1±0.6	1.2±0.4	1.5±0.8
Granulocytes (GRAN), 10^9/L	2.9±0.5	3.7±1.1	4.4±1.5	4.9±1.4*
Erythrocyte count (RBC), 10^{12}/L	7.07±0.72	7.26±0.22	6.99±0.69	7.38±0.23
Hemoglobin concentration (HGB), g/L	121.0±15.4	123.8±6.0	125.5±14.9	128.5±4.7
Hematocrit (HCT), %	28.9±3.0	29.6±1.1	29.3±3.2	31.5±1.2
Average erythrocyte volume (MCV), fL	40.9±0.9	40.8±0.4	41.9±1.1	42.7±0.7*
Mean corpuscular hemoglobin (MCH), pg	17.0±0.6	17.0±0.3	17.9±0.7	17.4±0.3
Mean corpuscular hemoglobin concentration (MCHC), g/dL	417.8±19.1	418.4±7.7	428.2±21.0	407.8±5.0
Thrombokrit (PCT), %	0.16±0.02	0.16±0.03	0.13±0.03	0.17±0.03

*- differences with the control group are statistically significant ($p < 0.05$); the Kruskal-Wallis test for independent samples was used to assess the significance of differences between control and experimental groups.

Table S14. Influence of daily i.v. administrations of Hep-IONPs on the blood biochemical parameters of rats (M±SD), on 14th day

Parameter	Experimental groups			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
14 days, male				

Alanine transaminase (ALT),	75.11±18.21	79.88±11.88	103.39±13.84	77.27±16.06
Aspartate transaminase (AST),	211.3±35.5	221.7±23.2	227.8±28.5	219.8±20.6
Alkaline phosphatase (AP), U/L	587.8±76.6	445.8±80.8	450.7±92.4	416.8±152.9
Total protein, g/L	66.54±2.07	69.13±5.45	68.54±3.34	68.17±3.74
Albumin, g/L	32.05±1.23	34.33±1.71	33.76±1.17	33.35±1.03
Glucose, mmol/L	5.52±0.51	5.87±0.69	6.34±0.57	5.77±0.71
Total cholesterol, mmol/L	1.62±0.18	1.89±0.26	1.76±0.29	1.64±0.33
Triglycerides,mmol/L	0.66±0.22	0.64±0.19	0.70±0.17	0.61±0.19
Urea, mmol/L	6.51±1.09	7.47±1.71	6.80±1.13	6.71±1.18
Creatinine, μmol/L	61.75±6.42	62.69±5.25	50.05±20.77	62.93±4.69
Total bilirubin, μmol/L	13.77±0.95	13.63±0.89	13.72±0.58	13.29±0.32
14 days, female				
Alanine transaminase (ALT),	61.98±11.15	70.35±13.69	59.92±13.69	61.79±11.63
Aspartate transaminase (AST),	212.0±30.1	227.7±33.4	198.0±44.8	188.3±26.1
Alkaline phosphatase (AP), U/L	355.5±87.7	333.0±83.8	457.8±189.0	284.7±39.7
Total protein, g/L	69.36±1.26	70.03±2.81	68.78±4.27	68.76±3.67
Albumin, g/L	34.28±1.12	34.96±1.32	33.27±2.75	34.16±1.35
Glucose, mmol/L	5.93±0.33	5.87±0.58	6.17±0.81	5.50±0.49
Total cholesterol, mmol/L	1.69±0.24	1.84±0.31	1.75±0.50	1.88±0.21
Triglycerides, mmol/L	0.75±0.16	0.72±0.12	0.57±0.11	0.84±0.27
Urea, mmol/L	7.18±0.59	6.99±0.98	8.52±1.86	8.16±0.87
Creatinine, μmol/L	64.45±10.92	62.32±13.29	53.09±11.16	55.61±9.86
Total bilirubin, μmol/L	6.70±1.18	7.72±0.82	7.62±1.11	7.14±0.27

*- differences with the control group are statistically significant ($p < 0.05$); the Kruskal-Wallis test for independent samples was used to assess the significance of differences between control and experimental groups.

Table S15. Influence of daily i.v. administrations of Hep-IONPs on the blood biochemical parameters of rats (M±SD), on 44th day

Parameter	Experimental group			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
30 days after the last injection, male				
Alanine transaminase (ALT),	89.80±16.06	81.75±7.16	102.88±34.95	80.83±8.76
Aspartate transaminase (AST),	228.0±37.8	239.3±47.5	232.5±31.1	216.2±24.9
Alkaline phosphatase (AP), U/L	313.3±72.3	294.8±67.95	326.8±83.6	270.3±85.1
Total protein, g/L	69.84±2.45	70.36±1.93	71.29±2.19	69.70±3.96
Albumin, g/L	35.19±0.66	35.33±1.08	35.14±0.42	34.24±1.41
Glucose, mmol/L	6.44±0.60	6.21±0.65	7.20±0.96	5.43±0.80
Total cholesterol, mmol/L	1.55±0.14	1.77±0.32	1.74±0.27	1.66±0.26
Triglycerides, mmol/L	0.67±0.15	0.79±0.19	0.57±0.20	0.81±0.14
Urea, mmol/L	9.0±0.61	875±1.05	8.54±0.91	9.64±1.10
Creatinine, μmol/L	39.91±12.72	36.87±8.17	39.64±8.62	33.03±10.21
Total bilirubin, μmol/L	8.54±0.84	7.06±0.53	7.83±1.44	6.71±0.83*
30 days after the last injection, female				
Alanine transaminase (ALT),	57.62±6.26	57.55±7.79	53.67±6.54	54.51±7.16
Aspartate transaminase (AST),	205.0±52.6	207.8±36	185.3±45.9	190.2±49.3
Alkaline phosphatase (AP), U/L	168.0±41.5	448.3±638.2	167.5±70.3	173.7±22.7
Total protein, g/L	65.06±2.33	68.63±3.42	67.20±3.18	69.93±5.04
Albumin, g/L	33.82±1.10	35.52±1.04	35.56±2.02	35.61±2.12
Glucose, mmol/L	6.23±0.95	6.70±0.56	7.68±0.89	7.82±0.78*

Total cholesterol, mmol/L	1.85±0.32	4.64±6.89	1.70±0.25	1.75±0.27
Triglycerides, mmol/L	0.62±0.09	0.75±0.19	0.69±0.21	0.86±0.21
Urea, mmol/L	10.15±1.19	10.51±1.99	8.93±0.53	9.16±0.58
Creatinine, μmol/L	61.64±12.56	62.79±18.47	68.45±13.38	65.15±10.81
Total bilirubin, μmol/L	7.34±0.91	7.17±1.53	7.56±0.56	7.17±0.82

*- differences with the control group are statistically significant ($p < 0.05$); the Kruskal-Wallis test for independent samples was used to assess the significance of differences between control and experimental groups.

Table S16. Influence of daily i.v. administrations of Hep-IONPs on the blood biochemical parameters of rabbits (M±SD), on 14th day

Parameter	Experimental groups			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
14 days, male				
Alanine transaminase (ALT),	35,99±8,02	52,74±21,36	34,68±9,46	46,56±8,49
Aspartate transaminase (AST),	24,3±12,4	22,3±1,5	19,7±5,5	16,0±6,2
Alkaline phosphatase (AP), U/L	420,3±70,9	489,7±164,3	497,3±120,7	487,0±111,5
Total protein, g/L	54,23±4,18	53,98±2,80	53,36±1,64	56,29±4,27
Albumin, g/L	32,30±2,04	32,60±1,80	33,37±1,05	34,77±2,87
Glucose, mmol/L	5,04±0,92	4,39±0,35	4,32±0,30	4,46±0,27
Total cholesterol, mmol/L	1,35±0,52	1,26±0,07	1,18±0,09	1,13±0,18
Triglycerides,mmol/L	1,08±0,64	1,11±0,28	1,47±0,74	0,75±0,17
Urea, mmol/L	5,81±1,32	6,19±0,45	5,85±0,21	6,18±1,10
Creatinine, μmol/L	77,39±17,85	84,67±2,56	83,32±7,15	90,80±8,50
Total bilirubin, μmol/L	6,03±0,61	6,39±0,33	5,93±0,34	5,95±1,12
14 days, female				
Alanine transaminase (ALT),	54,62±11,71	50,76±16,87	50,05±4,82	37,13±4,11
Aspartate transaminase (AST),	17,7±4,7	18,0±7,2	16,7±6,4	17,7±1,5
Alkaline phosphatase (AP), U/L	447,7±12,7	410,7±65,5	544,0±29,7	442,7±56,2
Total protein, g/L	56,27±3,11	55,85±5,13	60,88±1,14	56,83±1,78
Albumin, g/L	34,10±1,31	34,05±2,97	35,68±1,98	34,47±0,58
Glucose, mmol/L	4,88±0,33	4,93±0,52	4,67±0,13	4,95±0,38
Total cholesterol, mmol/L	1,54±0,33	1,87±0,24	2,23±0,13	1,49±0,20
Triglycerides, mmol/L	0,63±0,25	0,86±0,29	1,14±0,67	0,76±0,21
Urea, mmol/L	7,19±0,75	6,63±1,18	7,00±1,03	7,11±0,79
Creatinine, μmol/L	91,88±21,07	93,84±8,30	92,21±10,70	81,84±13,42
Total bilirubin, μmol/L	6,39±0,20	6,28±0,14	6,12±0,36	6,70±0,58

*- differences with the control group are statistically significant ($p < 0.05$); the Kruskal-Wallis test for independent samples was used to assess the significance of differences between control and experimental groups.

Table S17. Influence of daily i.v. administrations of Hep-IONPs on the blood biochemical parameters of rabbits (M±SD), on 44th day

Parameter	Experimental group			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
30 days after the last injection, male				
Alanine transaminase (ALT),	57,25±18,57	87,35±12,91	55,52±7,39	58,93±8,15
Aspartate transaminase (AST),	44,0±23,6	35,0±10,8	26,3±6,8	25,0±3,5
Alkaline phosphatase (AP), U/L	378,7±49,6	542,7±167,4	351,3±191,3	363,3±48,5

Total protein, g/L	63,92±1,13	60,92±2,05	63,77±4,74	60,20±4,13
Albumin, g/L	38,48±1,23	37,64±1,79	37,90±1,95	38,34±1,06
Glucose, mmol/L	6,28±0,81	5,73±0,15	6,27±0,61	5,94±0,38
Total cholesterol, mmol/L	1,22±0,11	0,88±0,21	1,30±0,07	1,45±0,37
Triglycerides, mmol/L	0,50±0,13	0,46±0,13	0,75±0,17	0,49±0,10
Urea, mmol/L	10,73±1,57	9,76±0,49	11,21±2,14	10,91±2,56
Creatinine, μmol/L	99,90±28,15	70,78±31,87	79,34±13,86	111,43±38,2
Total bilirubin, μmol/L	5,30±1,65	6,78±0,33	6,65±0,29	6,78±1,04
30 days after the last injection, female				
Alanine transaminase (ALT),	71,72±36,95	58,71±25,83	83,71±5,75	56,41±11,98
Aspartate transaminase (AST),	38,7±16,2	25,7±8,0	59,0±26,2	22,0±4,4
Alkaline phosphatase (AP), U/L	540,3±99,8	443,7±179,9	526,0±96,0	425,7±85,6
Total protein, g/L	65,858,17±	64,64±1,52	61,25±2,71	63,71±1,50
Albumin, g/L	49,15±20,56	40,68±0,60	40,15±0,88	40,26±2,63
Glucose, mmol/L	6,15±1,08	5,30±0,44	6,80±0,50	6,45±0,76
Total cholesterol, mmol/L	1,65±0,20	1,42±0,24	1,25±0,11	1,33±0,42
Triglycerides, mmol/L	0,70±0,13	0,61±0,11	0,51±0,12	0,53±0,11
Urea, mmol/L	12,22±0,87	12,57±0,43	11,13±1,61	11,39±1,03
Creatinine, μmol/L	71,25±28,89	86,69±10,24	64,31±18,15	71,86±14,77
Total bilirubin, μmol/L	7,09±0,87	6,08±0,22	5,83±1,07	6,14±0,84

*- differences with the control group are statistically significant ($p < 0.05$); the Kruskal-Wallis test for independent samples was used to assess the significance of differences between control and experimental groups.

Table S18. Influence of daily i.v. administrations of Hep-IONPs on the urinary system of male rats (M ± SD)

Parameter	Parameter			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Baseline values, males				
Urine output, mL	1.67±0.98	2.38±0.59	2.08±1.20	2.08±1.24
Color	Light yellow			
Transparency	Transparent			
Leukocytes, cells/μL	2.58±6.09	2.50±6.12	0.00±0.00	2.50±6.12
Ketone bodies, mmol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nitrite	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Urobilinogen	0.00±0.00	5.50±13.47	0.00±0.00	0.00±0.00
Bilirubin, μmol/L	1.43±3.51	5.50±13.47	0.00±0.00	1.43±3.51
Protein, g/L	0.46±0.43	0.29±0.37	0.24±0.39	0.10±0.16
Glucose, mmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Specific gravity	1.01±0.00	1.01±0.00	1.01±0.00	1.01±0.00
Blood erythrocytes/μL	15.8±10.7	40.8±78.5	11.7±11.3	12.5±13.7
pH	8.30±0.30	8.30±0.30	8.40±0.20	8.30±0.26
Ascorbic acid, mmol/L	0.00±0.00	0.10±0.20	0.00±0.00	0.00±0.00
14 days, males				
Urine output, mL	4.13±0.70	4.06±1.04	4.25±1.64	4.68±1.09
Color	Light yellow			
Transparency	Transparent			
Leukocytes, cells/μL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Ketone bodies, mmol	0.00±0.00	0.00±0.00	0.08±0.20	0.00±0.00
Nitrite	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Urobilinogen	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Bilirubin, μmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Protein, g/L	0.03±0.06	0.13±0.11	0.15±0.13	0.03±0.06
Glucose, mmol/L	0.00±0.00	0.00±0.00	0.47±0.47	0.00±0.00
Specific gravity	1.01±0.00	1.01±0.00	1.01±0.00	1.01±0.00
Blood erythrocytes/μL	43.3±28.4	38.3±79.4	81.7±63.9	77.5±69.0
pH	8.30±0.30	8.40±0.20	8.30±0.30	8.50±0.00
Ascorbic acid, mmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table S19. Influence of daily i.v. administrations of Hep-IONPs on the urinary system of female rats (M ± SD)

Parameter	Parameter			
	Control	1.4 mg/kg	7 mg/kg	14 mg/kg
Baseline values, females				
Urine output, mL	2.88±0.70	2.92±0.86	3.21±1.62	4.17±1.29
Color	Light yellow			
Transparency	Transparent			
Leukocytes, cells/μL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ketone bodies, mmol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nitrite	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Urobilinogen	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Bilirubin, μmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Protein, g/L	0.03±0.06	0.05±0.12	0.00±0.00	0.00±0.00
Glucose, mmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Specific gravity	1.01±0.00	1.01±0.00	1.01±0.00	1.01±0.00
Blood erythrocytes/μL	10.0±12.2	16.7±31.4	4.20±10.2	4.20±10.2
pH	7.70±0.50	7.6±0.7	7.60±0.7	7.70±0.40
Ascorbic acid, mmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
14 days, females				
Urine output, mL	2.58±1.28	2.92±1.02	2.67±0.61	4.17±0.61
Color	Light yellow			
Transparency	Transparent			
Leukocytes, cells/μL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ketone bodies, mmol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nitrite	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Urobilinogen	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Bilirubin, μmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Protein, g/L	0.03±0.06	0.05±0.12	0.03±0.06	0.00±0.00
Glucose, mmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Specific gravity	1.01±0.00	1.01±0.00	1.01±0.00	1.01±0.00
Blood erythrocytes/μL	1.70±4.10	3.30±5.20	48.3±80.6	0.00±0.00
pH	7.10±0.90	7.80±0.50	8.20±0.30	8.00±0.50
Ascorbic acid, mmol/L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

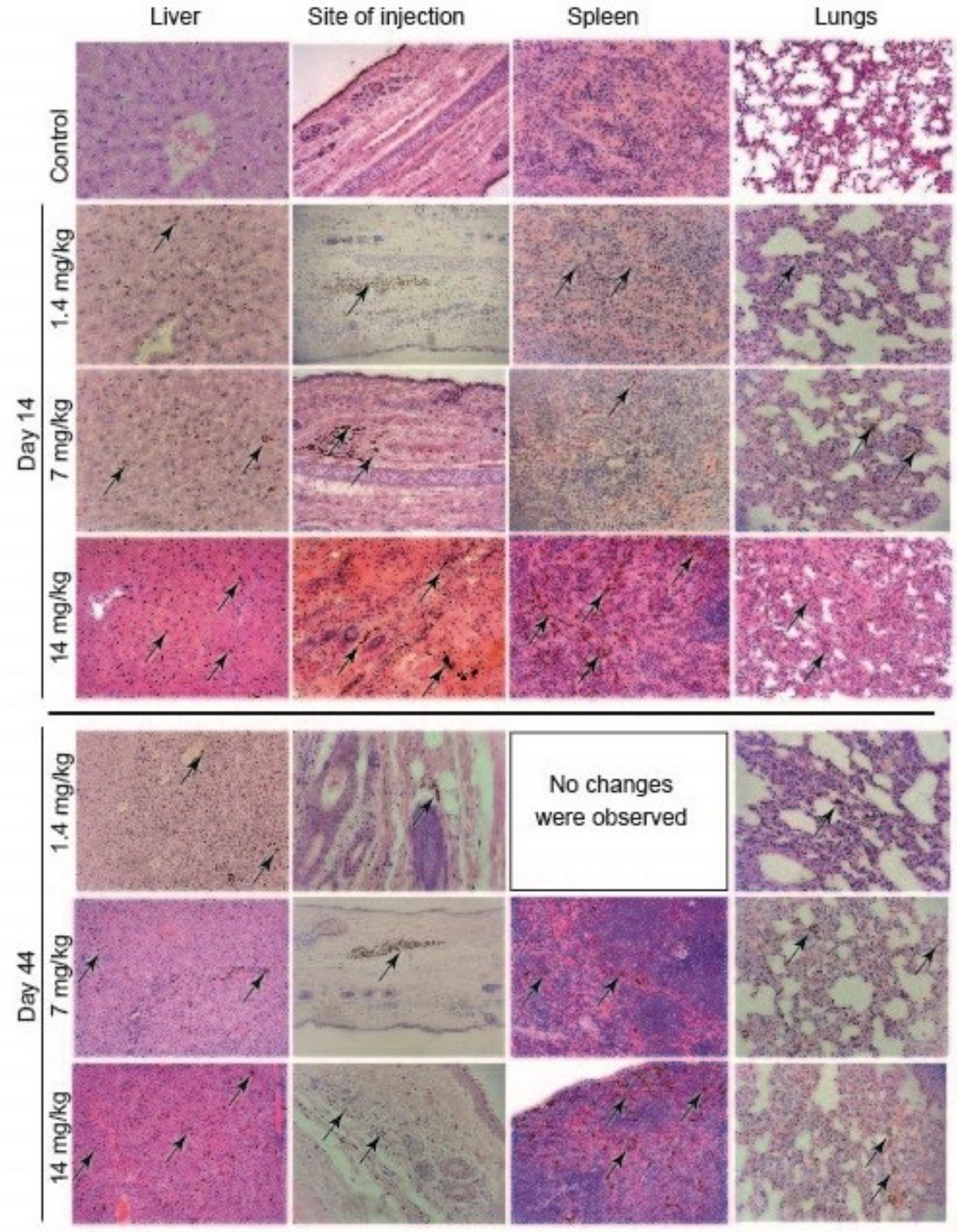

Figure S8. Images of histological sections of liver, spleen, lungs, and injection site of rabbits. Samples were collected after 14 days of injections and 30 days after the last injection. Sections were stained with H&E and observed under a light microscope at ×100 or ×200 magnification. Arrows show diffuse focal clusters of macrophages with Hep-IONPs in the cytoplasm