Bioavailability Study

Table S1. % HRE calculation for Nanoparticle Ferric pyrophosphate [NP-Fe$_4$(P$_2$O$_7$)$_3$] treated group

Rat No.	Initial Body Weight (g)	Final Body Weight (g)	Initial Hb (g/l)	Final Hb (g/l)	Initial mg of Fe in Hb	Final mg of Fe in Hb	Fe consumed (mg)	% HRE
1DFP10	109	140	45.6	90.2	1.11	2.83	1.42	121.03
2DFP10	70	106	50.4	102.4	0.79	2.43	1.16	141.75
3DFP10	153	177	45.0	100.5	1.54	3.99	1.7	143.95
4DFP10	73	93	56.4	90.0	0.92	1.87	1.13	84.47
5DFP10	117	148	58.6	77.0	1.53	2.55	1.59	64.08
6DFP10	125	123	59.0	110.8	1.65	3.05	1.54	91.14
7DFP10	79	103	52.0	82.0	0.92	1.89	1.53	63.63
8DFP10	70	114	54.1	81.8	0.84	2.09	1.37	90.73
9DFP20	56	81	54.9	73.9	0.69	1.34	2.58	25.32
10DFP20	121	157	63.0	83.0	1.71	2.92	3.20	37.93
11DFP20	96	132	59.0	105.3	1.27	3.11	3.10	59.62
12DFP20	120	184	51.0	81.8	1.37	3.37	4.10	48.89
13DFP20	113	119	58.0	81.0	1.47	2.16	3.08	22.48
14DFP20	100	136	56.0	83.0	1.25	2.53	2.62	48.72
15DFP20	131	174	49.0	113.0	1.44	4.41	3.34	88.99
16DFP20	95	116	51.0	94.2	1.08	2.45	2.94	46.43
17DFP30	100	127	44.9	76.0	1.00	2.16	4.74	24.44

18DFP30	69	115	55.9	95.9	0.86	2.47	4.50	35.76
19DFP30	76	117	54.5	74.0	0.92	1.94	5.04	20.11
20DFP30	112	162	48.0	82.0	1.20	2.98	5.37	33.05
21DFP30	115	151	57.3	84.6	1.47	2.86	4.65	29.85
22DFP30	42	65	47.5	79.0	0.44	1.15	2.58	27.31
23DFP30	95	133	41.0	81.0	0.87	2.41	4.44	34.76
24DFP30	119	156	48.0	90.0	1.28	3.15	4.80	38.94
Average	98.1	130.3	52.5	88.0	1.15	2.58	3.02	59.30

DFP = Iron-depleted rats fed with NP-$Fe_4(P_2O_7)_3$ fortified food (at respective doses 10, 20, 30 mg/kg diet).

Table S2. % HRE calculation for FeSO$_4$ treated group

Rat No	Initial Body weight (g)	Final Body Weight (g)	Initial Hb (g/l)	Final Hb (g/l)	Initial mg of Fe in Hb	Final mg of Fe in Hb	Fe consumed (mg)	% HRE
25DFS10	122	163	50.0	78.0	1.36	2.85	2.03	73.12
26DFS10	54	73	62.0	77.0	0.75	1.26	1.29	39.54
27DFS10	92	152	59.0	136.0	1.21	4.63	1.63	209.90
28DFS10	90	118	53.0	82.0	0.16	2.17	1.65	121.58
29DFS10	70	93	53.0	70.0	0.83	1.46	1.34	46.90
30DFS10	123	154	41.0	81.6	1.13	2.82	1.60	105.53
31DFS10	77	105	59.3	94.7	1.02	2.23	1.70	70.99
32DFS10	114	125	59.8	75.0	1.53	2.10	1.74	32.99
33DFS20	92	135	51.8	94.0	1.06	2.84	2.78	63.97
34DFS20	53	55	46.0	71.0	0.54	0.87	1.86	17.70
35DFS20	136	167	45.0	85.0	1.37	3.18	3.92	46.23
36DFS20	93	123	58.0	112.0	1.21	3.09	3.24	58.06
37DFS20	103	129	58.1	80.8	1.34	2.33	2.62	38.02
38DFS20	78	80	54.0	73.7	0.94	1.32	2.4	15.74
39DFS20	92	125	60.0	97.1	1.23	2.72	2.94	50.52
40DFS20	104	158	61.0	118.0	1.42	4.18	3.72	74.21
41DFS30	98	130	54.0	107.6	1.18	3.13	4.92	39.67
42DFS30	103	125	72.0	107.4	1.66	3.01	4.08	33.05
43DFS30	130	160	56.0	108.0	1.63	3.87	5.31	42.26
44DFS30	90	118	58.1	104.0	1.17	2.75	4.11	38.46

45DFS30	93	130	58.0	104.0	1.21	3.03	4.77	38.23
46DFS30	97	121	55.2	109.0	1.20	2.96	4.23	41.57
47DFS30	68	114	44.0	106.0	0.67	2.71	4.56	44.75
48DFS30	93	124	53.0	108.1	1.10	3.0	4.92	38.66
Average	94.3	124.0	55.0	95.0	1.12	2.68	3.05	57.56

DFS = Iron-depleted rats fed with FeSO$_4$ fortified food (at respective doses 10, 20, 30 mg/kg diet).

Table S3. Change in hemoglobin level of rats during repletion period for NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Salt	No. of	Baseline (B)		No. of	Endline (E)		p-value
	rats	Mean	SD	rats	Mean	SD	B *vs* E
NP-Fe$_4$(P$_2$O$_7$)$_3$	24	5.25	0.558	24	8.80	1.136	0.000
FeSO$_4$	24	5.50	0.673	24	9.50	1.738	0.000

p – value from paired t test.

Table S4. Comparison of change in hemoglobin level between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Salt	No. of rats	Hb Difference		p-value between two groups
		Mean	SD	
NP-Fe$_4$(P$_2$O$_7$)$_3$	24	3.55	1.25	0.142
FeSO$_4$	24	3.99	1.63	

p – value from Independent t test.

Table S5. Comparison of liver enzyme values between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameters	NP-Fe$_4$(P$_2$O$_7$)$_3$		FeSO$_4$		p-value
	Median	q1, q2	Median	q1, q2	
ALT	23.42	18.56, 59.22	29.17	26.52, 77.70	0.088
AST	77.79	49.40, 97.68	55.69	38.39, 100.77	0.424
ALP	98.98	28.13, 187.46	118.65	48.13, 230.52	0.570

p – value from mixed model analysis.

Table S6. Comparison of biochemical parameters between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameters	NP-Fe$_4$(P$_2$O$_7$)$_3$	FeSO$_4$	p-value NP-Fe$_4$(P$_2$O$_7$)$_3$ *vs* FeSO$_4$
Albumin (g/dL)	3.45 ± 0.430	3.20 ± 0.3835	0.079
Creatinine (mg/dL)	0.62 ± 0.290	0.62 ± 0.142	0.170
Total protein (g/dL)	5.58 ± 1.750	5.71 ± 0.540	0.949
Glucose (mmol/L)	3.53 ± 2.32	4.12 ± 2.25	0.728

Values expressed in mean ± standard deviation, p – value from Independent sample t test.

Table S7. Comparison of hematological parameters between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameters	NP-Fe$_4$(P$_2$O$_7$)$_3$	FeSO$_4$	p-value NP-Fe$_4$(P$_2$O$_7$)$_3$ *vs* FeSO$_4$
RBC (x10^{6}µl)	5.89 ± 0.68	6.13 ± 0.81	[#]0.282
HCT (%)	20.72 ± 4.85	22.14 ± 3.84	[#]0.234
MCV (fl)	35.84 ± 5.40	36.24 ± 3.80	[#]0.606
MCH (pg)	10.93 ± 2.19	12.55 ± 4.70	[#]0.349
MCHC (g/dL)	30.51 ± 2.16	34.92 ± 13.04	[#]0.666
RDW (%)	26.66 ± 3.14	26.50 ± 3.09	[#]0.622
PLT (x10^{3}µl)	610.52 ± 357.09	577.309 ± 355.24	[*]0.707
PCT (%)	0.440 ± 0.24	0.420 ± 0.23	[*]0.991
MPV (fl)	6.86 ± 0.72	6.98 ± 0.96	[#]0.945

| PDW (fl) | 5.86 ± 1.19 | 5.88 ± 1.96 | #0.791 |

Values expressed in mean ± standard deviation, * p – value from Independent sample t test,
p – value from Mann-Whitney U test.

Table S8. Comparison of reduced glutathione between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameter	NP-Fe$_4$(P$_2$O$_7$)$_3$		FeSO$_4$		p-value
	Median	q1, q2	Median	q1, q2	
Reduced GSH	9.77	1.91, 16.09	14.02	10.01, 15.07	0.256

p – value from mixed model analysis.

Table S9. Comparison of grades of Prussian blue staining between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups for the organs spleen and bone marrow

Organs	NP-Fe$_4$(P$_2$O$_7$)$_3$			FeSO$_4$			p-value
	No. of rats	Median	q1,q2	No. of rats	Median	q1,q2	NP-Fe$_4$(P$_2$O$_7$)$_3$ vs FeSO$_4$
Spleen	24	1.00	0, 2.00	24	1.00	0, 3.00	0.945
Bone Marrow	24	3.00	2.00, 4.00	24	3.00	1.00, 4.00	0.642

p – value from mixed model analysis.

Acute Oral Toxicity Study

Table S10. Comparison of food intake, water consumption and body weight between control and NP-Fe$_4$(P$_2$O$_7$)$_3$ treated groups

Parameters	Control	NP-Fe$_4$(P$_2$O$_7$)$_3$	p-value
Food intake (gm)	13.19 ± 2.30	14.26 ± 2.55	0.635
Water Consumption (ml)	25.44 ± 5.71	23.60 ± 4.50	0.458
Body weight (gm)	239.88 ± 44.23	229.02 ± 35.35	0.385

Values expressed in mean ± standard deviation, p – value from Independent t test.

Table S11. Comparison of biochemical parameters between control and NP-Fe$_4$(P$_2$O$_7$)$_3$ treated groups

Parameters	Control	NP-Fe$_4$(P$_2$O$_7$)$_3$	p-value
Albumin (g/dL)	4.728 ± 0.54	4.546 ± 0.35	0.385[*]
Glucose (mmol/L)	7.040 ± 1.40	7.430 ± 0.98	0.486[*]
Total protein (g/dL)	7.626 ± 1.25	7.648 ± 1.29	0.969[*]
ALT (IU/L)	93.890 ± 28.82	106.860 ± 30.88	0.371[*]
Creatinine (mg/dL)	46.951 ± 14.41	53.432 ± 15.44	0.371[*]
Bilirubin (mg/dL)	0.166 ± 0.11	0.162 ± 0.09	0.869[#]
ALP (IU/L)	28.77 ± 12.09	29.696 ± 16.43	0.712[#]
AST (IU/L)	22.00 ± 13.92	15.910 ± 5.40	0.709[#]

Values expressed in mean ± standard deviation, [*] p – value from Independent sample t test, [#] p – value from Mann-Whitney U test.

Table S12. Comparison of hematological parameters between control and NP-Fe$_4$(P$_2$O$_7$)$_3$ treated groups

Parameters	Control	NP-Fe$_4$(P$_2$O$_7$)$_3$	p-value
RBC (x10^6/μl)	6.927 ± 1.08	7.009 ± 0.54	0.834[*]
Hb (g/dL)	13.070 ± 2.24	13.420 ± 1.54	0.698[*]
HCT (%)	49.220 ± 2.02	48.170 ± 1.99	0.270[*]
MCH (pg)	18.830 ± 0.75	18.920 ± 1.95	0.327[*]
MCHC (g/dl)	38.410 ± 1.70	39.270 ± 3.01	0.482[*]
RDW (%)	14.430 ± 2.53	13.650 ± 0.39	0.343[*]
PDW (fl)	9.160 ± 1.61	9.520 ± 1.30	0.609[*]
MPV (fl)	7.970 ± 0.51	8.190 ± 0.57	0.403[*]
MCV (fl)	20.630 ± 5.46	18.920 ± 1.95	0.269[#]
PLT(x10^{3}μl)	300.300 ± 134.40	358.100 ± 190.20	0.315[#]
PCT (%)	0.298 ± 0.19	0.259 ± 0.16	0.859[#]

Values expressed in mean ± standard deviation,[*]p-value from Independent sample t test,
[#] p – value from Mann-Whitney U test.

Table S13. Comparison of reduced glutathione between control and NP-Fe$_4$(P$_2$O$_7$)$_3$ treated groups

Parameter	Control	NP-Fe$_4$(P$_2$O$_7$)$_3$	p-value
Reduced Glutathione (mM)	2.304 ± 0.91	1.79 ± 0.57	0.171

Values expressed in mean ± standard deviation, p – value from Independent sample t test.

The comparison of Prussian blue staining grades of all the organs namely lungs, kidney, brain, liver, spleen, heart, pancreas, bone marrow, intestine and stomach for control and NP-Fe$_4$(P$_2$O$_7$)$_3$ treated group did not show any significant difference between the groups. The grades of Prussian blue stains showed grade 0 for all the organs of both the groups.

Repeated Oral Toxicity Study

Table S14. Comparison of food intake, water consumption and body weight between control (C), NP-Fe$_4$(P$_2$O$_7$)$_3$ (N) and micronized Fe$_4$(P$_2$O$_7$)$_3$ (M) treated groups

Parameters	C	N	M	p-value		
				N *vs* C	M *vs* C	N *vs* M
Food intake (gm)	9.55 ± 0.79	9.61 ± 0.39	9.94 ± 1.37	0.083	0.172	0.971
Water consumption (ml)	13.00 ± 0.77	12.79 ± 1.34	14.40 ± 1.59	0.184	0.251	0.889
Body weight (gm)	167.92 ± 4.86	163.62 ± 5.58	156.57 ± 10.79	0.930	0.067	0.094

Values expressed in mean ± standard deviation, p – value from Independent t test.

Table S15. Comparison of biochemical parameters among C, N and M treated groups

Parameters	C	N	M	*p-value			#p-value		
				N *vs* C	M *vs* C	N *vs* M	N *vs* C	M *vs* C	N *vs* M
Albumin (g/dL)	3.92 ± 0.58	3.65 ± 0.77	3.89 ± 0.63	----	----	----	0.431	0.93	0.488
Creatinine (mg/dL)	1.01 ± 0.22	1.12 ± 0.23		----	----	----	----	0.4	----	----

	C	N	M	*	*	*	#	#	#
ALT (IU/L)	30.05 ± 9.35	39.85 ± 6.33	----	----	----	----	0.099	----	----
AST (IU/L)	76.16 ± 24.97	157.74 ± 49.32	89.28 ± 37.59	0.014	0.655	0.157	----	----	----
ALP (IU/L)	39.51 ± 18.58	59 ± 21.02	55.59 ± 15.26	0.085	0.086	0.721	----	----	----
Total protein (g/dL)	5.93 ± 0.74	5.48 ± 0.32	----	----	----	----	0.209	----	----
Glucose (mmol/L)	5.13 ± 0.35	5.12 ± 0.52	6.14 ± 1.10	----	----	----	0.962	0.042	0.051

Values expressed in mean ± standard deviation, [*] p – value from Mann-Whitney U test

[#] p – value from Independent sample t test.

Table S16. Comparison of hematological parameters among C, N and M treated groups

Parameters	C	N	M	p-value		
				N *vs* C	M *vs* C	N *vs* M
Hb (g/dL)	15.90 ± 1.540	16.17 ± 2.19	16.210 ± 1.28	0.36	0.364	0.778
RBC (x10⁶μl)	7.45 ± 1.050	7.68 ± 0.94	7.877 ± 0.74	0.622	0.319	0.614
HCT (%)	38.08 ± 5.940	39.50 ± 6.10	40.910 ± 2.94	0.605	0.198	0.528
MCV (fl)	51.76 ± 2.670	51.60 ± 4.23	52.021 ± 2.41	0.945	0.83	0.786
MCH (pg)	18.81 ± 1.320	19.20 ± 1.08	19.100 ± 0.93	0.425	0.596	0.685
MCHC (g/dl)	36.62 ± 2.430	37.00 ± 1.23	36.750 ± 1.17	0.675	0.885	0.642

RDW (%)	13.46 ± 0.660	13.20 ± 0.67	13.570 ± 0.68	0.572	0.75	0.361
PLT (x10³μl)	459.17 ± 101.550	604.20 ± 231.70	552.330 ± 169.22	0.105	0.176	0.594
PCT (%)	0.36 ± 0.076	0.48 ± 0.20	0.440 ± 0.13	0.132	0.166	0.632
MPV (fl)	8.67 ± 1.180	8.75 ± 1.18	8.720 ± 1.03	0.879	0.925	0.945
PDW (fl)	9.88 ± 1.760	9.90 ± 1.43	10.940 ± 1.51	0.908	0.202	0.183

Values expressed in mean ± standard deviation, p – value from Independent t test.

Table S17. Comparison of reduced glutathione (GSH) among C, N and M treated groups

Groups	GSH Endline	*p-value	#p-value N *vs* C	#p-value M *vs* C	#p-value N *vs* M
Control	0.860 ± 0.15	0.042	0.502	0.499	0.879
Nano	0.920 ± 0.22	0.165	------	------	------
Micro	0.935 ± 0.30	0.356	------	------	------

Values expressed in mean ± standard deviation, * p – value from paired t test,
p – value from Independent t test.

Table S18. Comparison of grades of Prussian blue staining among C, N and M treated groups

Organs	Lungs	Kidney	Brain	Liver	Spleen	Heart	Pancreas	Bone marrow	Intestine	Stomach
p - value	0.169	0.459	1.000	0.505	0.128	0.229	1.000	0.094	0.403	1.000
Median value	0	0	0	0	2	0	0	0	0	0
Quartile										
25	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	2	0	0	0	0	0
75	2	0	0	2	4	0	0	2	0	0

p – value from analysis of variance (ANOVA).

Proteomics Study

Table S19. Comparison of liver enzyme between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameters	NP-Fe$_4$(P$_2$O$_7$)$_3$			FeSO$_4$			p-value
	No. of rats	Median	q1, q2	No. of rats	Median	q1, q2	
ALT	11	37.12	20.33, 46.85	14	31.38	17.68, 55.470	0.727
AST	17	65.62	54.80, 87.51	18	82.35	46.63,115.241	0.207
ALP	10	71.69	36.95, 97.29	11	92.20	43.39, 131.530	0.387

p – value from mixed model analysis.

Table S20. Comparison of biochemical parameters between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameters	NP-Fe$_4$(P$_2$O$_7$)$_3$	FeSO$_4$	p-value NP-Fe$_4$(P$_2$O$_7$)$_3$ vs FeSO$_4$
Albumin (g/dL)	3.48 ± 0.492	3.51 ± 0.713	*0.122
Creatinine (mg/dL)	0.68 ± 0.220	0.67 ± 0.199	*0.659
Total protein (g/dL)	5.23 ± 1.140	5.65 ± 1.310	*0.554
Glucose (mmol/L)	5.00 ± 1.610	3.79 ± 1.390	#0.573

Values expressed in mean ± standard deviation, * p – value from Mann-Whitney U test

p – value from Independent sample t test.

Table S21. Comparison of hematological parameters between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameters	NP-Fe$_4$(P$_2$O$_7$)$_3$	FeSO$_4$	p-value NP-Fe$_4$(P$_2$O$_7$)$_3$ *vs* FeSO$_4$
Hb (g/dL)	12.21 ± 1.64	12.980 ± 1.650	0.439
RBC (x10^{6}µl)	7.25 ± 0.48	7.407 ± 0.880	0.526
HCT (%)	32.82 ± 2.44	34.760 ± 6.010	0.664
MCV (fl)	45.05 ± 2.57	45.970 ± 8.710	0.240
MCH (pg)	17.47 ± 3.83	18.120 ± 5.900	0.201
MCHC (g/dL)	37.21 ± 2.23	35.970 ± 5.200	0.760
RDW (%)	18.89 ± 2.37	19.220 ± 3.480	0.716
PLT (x10^{3}µl)	633 ± 215	647.220 ± 342	0.270
PCT (%)	0.464 ± 0.15	0.509 ± 0.230	0.842
MPV (fl)	7.610 ± 1	7.950 ± 0.819	0.110
PDW (fl)	8.380 ± 2.06	8.860 ± 1.170	0.327

Values expressed in mean ± standard deviation, p – value from Independent t test.

Table S22. Comparison of reduced glutathione between NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups

Parameter	NP-Fe$_4$(P$_2$O$_7$)$_3$		FeSO$_4$		p-value
	Median	q1, q2	Median	q1, q2	
Reduced GSH	8.47	8.12, 9.8	8.47	8.12, 9.8	0.926

p – value from mixed model analysis.

Table S23. Comparison of grades of Prussian blue staining among NP-Fe$_4$(P$_2$O$_7$)$_3$ and FeSO$_4$ treated groups for the organs spleen and bone marrow

Organs	NP-Fe$_4$(P$_2$O$_7$)$_3$			FeSO$_4$			p-value
	No. of rats	Median	q1,q2	No. of rats	Median	q1,q2	NP-Fe$_4$(P$_2$O$_7$)$_3$ vs FeSO$_4$
Spleen	24	2.00	0, 3.00	24	1.00	0, 3.00	0.982
Bone Marrow	24	3.00	2.00, 4.00	24	3.00	1.00, 4.00	1.000

p – value from mixed model analysis