Polyphenol Effects on CuO-Nanoparticle-Mediated DNA Damage, Reactive Oxygen Species Generation, and Fibroblast Cell Death

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SUPPLEMENTARY MATERIAL

DNA gel data and IC_{50} plots. Gel electrophoresis images of plasmid DNA treated with various polyphenols and IC_{50} plots are presented in Figures S1-S10. All data are the average of three independent experiments with error bars corresponding to calculated standard deviations. Data for resulting DNA band intensities are provided in Tables S1-S10. Graphs showing lack of correlation of IC_{50} values with polyphenol oxidation potential and pK_a of the first phenolic hydrogen are given in Figure S11.

Electron paramagnetic resonance studies. Electron paramagnetic resonance (EPR) spectra for the detection and identification of reactive oxygen species (ROS) generated by ^{NP}CuO with ascorbic acid and H_2O_2 are shown in Figures S12 and S13. Spectra were recorded in buffered aqueous solutions (pH 6.3) at room temperature with the following experimental parameters: time constant 81.92 ms, conversion time 81.92 ms, modulation amplitude 1.00 G, microwave power 20.02, and magnetic field 3500 ± 100 G, unless otherwise indicated.

Dynamic light scattering measurements. Dynamic light scattering (DLS) measurements of ^{NP}CuO and washed CuO nanoparticles (^{w}CuO) in water and Eagle's minimum essential medium (EMEM) are presented in Table S11 and Figure S14. Data are reported as means of at least three trials with standard deviations for the errors.

Cell data and EC_{50} plots. Cell viability data and EC_{50} plots of L929 cells treated with ^{NP}CuO, washed CuO nanoparticles (^{w}CuO), ^{NP}CuO supernatant with dissolved copper removed (^{l}CuO), and dissolved copper ($CuCl_2$) solutions with or without H_2O_2, MEPCA, and MEGA for 24 h are presented in Figures S15-S22 and Tables S12-S19. EC_{50} values are reported with standard deviations for the errors and were calculated by fitting all points of four trials with a single curve.

Table S1. DNA damage inhibition by methyl-3,4-dihydroxybenzoate (MEPCA; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[MEPCA] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	97.3 ± 1.4	2.7 ± 1.4	-
2	p + H_2O_2 (50 μM)	0	95.1 ± 1.8	4.9 ± 1.8	-
3	p + MEPCA (800 μM)	800	95.7 ± 1.3	4.3 ± 1.3	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	9.3 ± 6.1	90.7 ± 6.1	-
5	p + ^{NP}CuO + H_2O_2 + MEPCA	0.5	17.0 ± 4.5	83.0 ± 4.5	0.0975
6	p + ^{NP}CuO + H_2O_2 + MEPCA	1	31.7 ± 3.2	68.3 ± 3.2	0.0067
7	p + ^{NP}CuO + H_2O_2 + MEPCA	2	34.0 ± 2.6	66.0 ± 2.6	0.0037
8	p + ^{NP}CuO + H_2O_2 + MEPCA	5	43.5 ± 4.0	56.5 ± 4.0	0.0045
9	p + ^{NP}CuO + H_2O_2 + MEPCA	10	66.5 ± 4.7	33.5 ± 4.7	0.0022
10	p + ^{NP}CuO + H_2O_2 + MEPCA	25	83.6 ± 0.8	16.4 ± 0.8	<0.0001
11	p + ^{NP}CuO + H_2O_2 + MEPCA	50	86.8 ± 3.3	13.2 ± 3.3	0.0006
12	p + ^{NP}CuO + H_2O_2 + MEPCA	100	92.8 ± 2.4	7.2 ± 2.4	0.0003
13	p + ^{NP}CuO + H_2O_2 + MEPCA	200	95.4 ± 2.4	4.6 ± 2.4	0.0003
14	p + ^{NP}CuO + H_2O_2 + MEPCA	400	98.9 ± 0.9	1.1 ± 0.9	<0.0001
15	p + ^{NP}CuO + H_2O_2 + MEPCA	800	99.9 ± 0.1	0.1 ± 0.1	<0.0001

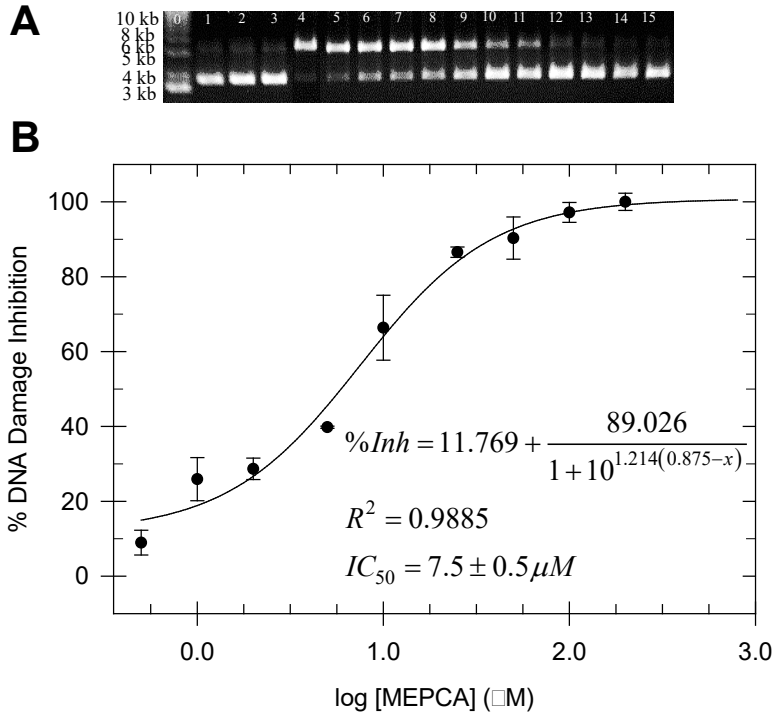

Figure S1. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and MEPCA (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + MEPCA (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of MEPCA (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to MEPCA concentration in the presence of ^{NP}CuO and H_2O_2.

Table S2. DNA damage inhibition by propyl gallate (PREGA; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[PREGA] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	99.7 ± 0.0	0.3 ± 0.0	-
2	p + H_2O_2 (50 μM)	0	98.3 ± 0.3	1.7 ± 0.3	-
3	p + PREGA (800 μM)	1200	93.3 ± 3.1	6.7 ± 3.1	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	2.9 ± 5.0	97.1 ± 5.0	-
5	p + ^{NP}CuO + H_2O_2 + PREGA	0.5	5.5 ± 5.4	94.5 ± 5.4	0.492
6	p + ^{NP}CuO + H_2O_2 + PREGA	1	4.6 ± 3.0	95.4 ± 3.0	0.4298
7	p + ^{NP}CuO + H_2O_2 + PREGA	2	2.1 ± 2.9	97.9 ± 2.9	0.6799
8	p + ^{NP}CuO + H_2O_2 + PREGA	5	3.1 ± 4.6	96.9 ± 4.6	0.9468
9	p + ^{NP}CuO + H_2O_2 + PREGA	10	5.5 ± 4.2	94.5 ± 4.2	0.3958
10	p + ^{NP}CuO + H_2O_2 + PREGA	25	5.9 ± 5.1	94.1 ± 5.1	0.4155
11	p + ^{NP}CuO + H_2O_2 + PREGA	50	37.5 ± 3.2	62.5 ± 3.2	0.0028
12	p + ^{NP}CuO + H_2O_2 + PREGA	100	45.8 ± 6.9	54.2 ± 6.9	0.0085
13	p + ^{NP}CuO + H_2O_2 + PREGA	200	66.3 ± 9.6	33.7 ± 9.6	0.0076
14	p + ^{NP}CuO + H_2O_2 + PREGA	400	78.8 ± 1.4	21.2 ± 1.4	0.0001
15	p + ^{NP}CuO + H_2O_2 + PREGA	800	89.0 ± 1.7	11.0 ± 1.7	0.0001
16	p + ^{NP}CuO + H_2O_2 + PREGA	1200	96.4 ± 0.9	3.6 ± 0.9	<0.0001

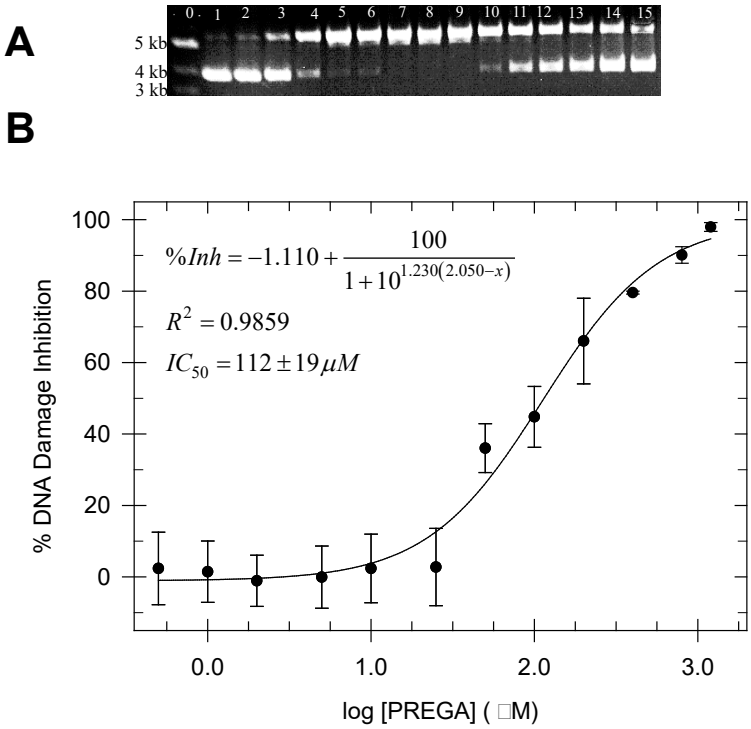

Figure S2. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and PREGA (0.5 – 1200 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + PREGA (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of PREGA (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, 800, and 1200 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to PREGA concentration in the presence of ^{NP}CuO and H_2O_2.

Table S3. DNA damage inhibition by epicatechin gallate (ECG; 0.5 – 1200 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[ECG] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	97.5 ± 4.4	2.5 ± 4.4	-
2	p + H_2O_2 (50 μM)	0	96.2 ± 2.9	3.8 ± 2.9	-
3	P + ECG (800 μM)	1200	93.2 ± 4.4	6.8 ± 4.4	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	0.5 ± 0.4	99.5 ± 0.4	-
5	p + ^{NP}CuO + H_2O_2 + ECG	0.5	0.7 ± 1.2	99.3 ± 1.2	0.8
6	p + ^{NP}CuO + H_2O_2 + ECG	1	0.1 ± 0.1	99.9 ± 0.1	0.0202
7	p + ^{NP}CuO + H_2O_2 + ECG	2	0.1 ± 0.1	99.9 ± 0.1	0.0202
8	p + ^{NP}CuO + H_2O_2 + ECG	5	0.1 ± 0.0	99.9 ± 0.0	>0.9999
9	p + ^{NP}CuO + H_2O_2 + ECG	10	2.5 ± 2.7	97.5 ± 2.7	0.3281
10	p + ^{NP}CuO + H_2O_2 + ECG	25	4.3 ± 3.4	95.7 ± 3.4	0.1925
11	p + ^{NP}CuO + H_2O_2 + ECG	50	26.8 ± 6.0	73.2 ± 6.0	0.0169
12	p + ^{NP}CuO + H_2O_2 + ECG	100	43.6 ± 4.3	56.4 ± 4.3	0.0033
13	p + ^{NP}CuO + H_2O_2 + ECG	200	51.7 ± 4.4	48.3 ± 4.4	0.0025
14	p + ^{NP}CuO + H_2O_2 + ECG	400	68.2 ± 4.3	31.8 ± 4.3	0.0013
15	p + ^{NP}CuO + H_2O_2 + ECG	800	75.9 ± 2.2	24.1 ± 2.2	0.0003
16	p + ^{NP}CuO + H_2O_2 + ECG	1200	85.4 ± 2.4	14.6 ± 2.4	0.0003

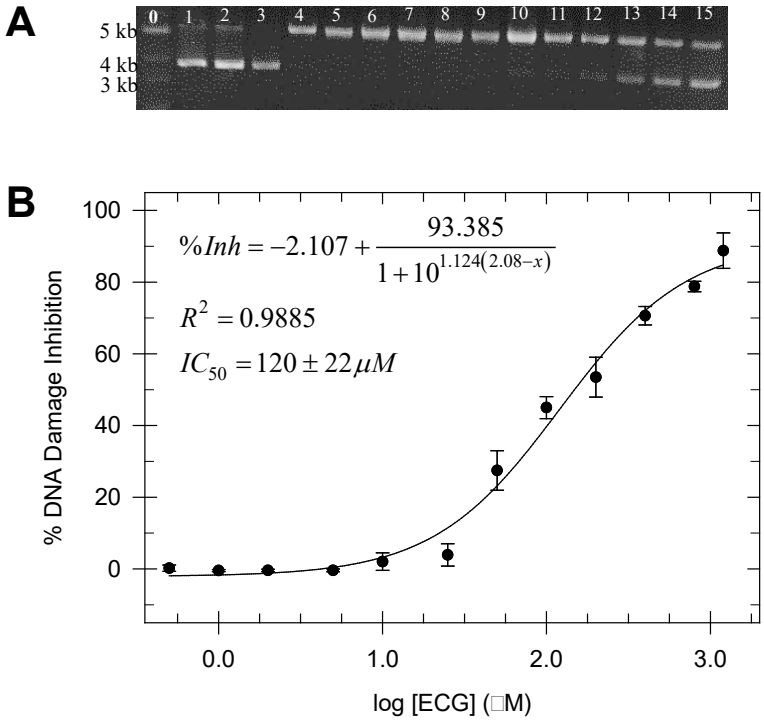

Figure S3. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and ECG (0.5 – 1200 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + ECG (1200 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5 -16: p + ^{NP}CuO (500 μM) + increasing concentrations of ECG (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, 800, and 1200 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to ECG concentration in the presence of ^{NP}CuO and H_2O_2.

Table S4. DNA damage inhibition by methyl-3,4,5-trihydroxybenzoate (MEGA; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[MEGA] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	96.6 ± 2.5	3.4 ± 2.5	-
2	p + H_2O_2 (50 μM)	0	96.1 ± 1.3	3.9 ± 1.3	-
3	p + MEGA (800 μM)	800	96.4 ± 1.1	3.6 ± 1.1	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	5.3 ± 2.9	94.7 ± 2.9	-
5	p + ^{NP}CuO + H_2O_2 + MEGA	0.5	10.1 ± 5.3	89.9 ± 5.3	0.2573
6	p + ^{NP}CuO + H_2O_2 + MEGA	1	4.5 ± 3.4	95.5 ± 3.4	0.7231
7	p + ^{NP}CuO + H_2O_2 + MEGA	2	1.8 ± 2.9	98.2 ± 2.9	0.1717
8	p + ^{NP}CuO + H_2O_2 + MEGA	5	0.1 ± 0.1	99.9 ± 0.1	0.0001
9	p + ^{NP}CuO + H_2O_2 + MEGA	10	0.2 ± 0.1	99.8 ± 0.1	0.0001
10	p + ^{NP}CuO + H_2O_2 + MEGA	25	1.0 ± 0.8	99.0 ± 0.8	0.0113
11	p + ^{NP}CuO + H_2O_2 + MEGA	50	16.0 ± 4.5	84.0 ± 4.5	0.0542
12	p + ^{NP}CuO + H_2O_2 + MEGA	100	35.8 ± 2.2	64.2 ± 2.2	0.0017
13	p + ^{NP}CuO + H_2O_2 + MEGA	200	51.5 ± 0.8	48.5 ± 0.8	<0.0001
14	p + ^{NP}CuO + H_2O_2 + MEGA	400	74.9 ± 3.7	25.1 ± 3.7	0.0009
15	p + ^{NP}CuO + H_2O_2 + MEGA	800	86.9 ± 1.9	13.1 ± 1.9	0.0002
16	p + ^{NP}CuO + H_2O_2 + MEGA	1200	97.2 ± 2.2	2.8 ± 2.2	0.0002

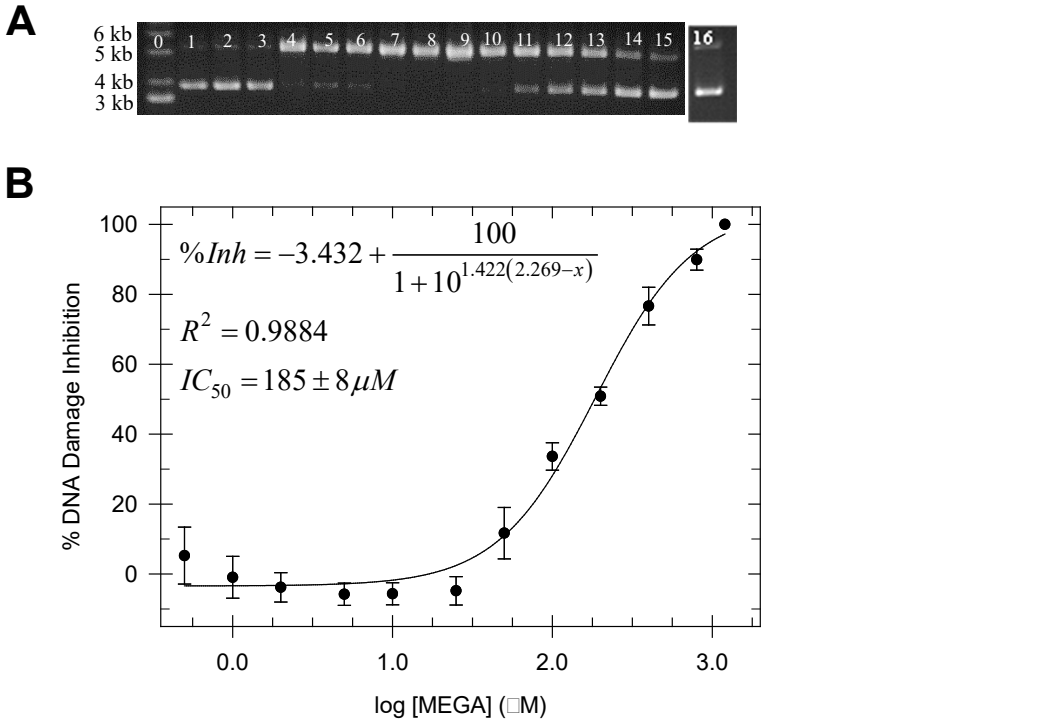

Figure S4. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) and MEGA (0.5 – 1200 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + MEGA (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5 -16: p + ^{NP}CuO (500 μM) + increasing concentrations of MEGA (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, 800, and 1200 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to MEGA concentration in the presence of ^{NP}CuO and H_2O_2.

Table S5. DNA damage inhibition by epigallocatechin gallate (EGCG; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[EGCG] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	98.6 ± 1.3	1.4 ± 1.3	-
2	p + H_2O_2 (50 μM)	0	98.6 ± 0.6	1.4 ± 0.6	-
3	p + EGCG (800 μM)	800	98.1 ± 1.9	1.9 ± 1.9	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	5.3 ± 7.0	94.7 ± 7.0	-
5	p + ^{NP}CuO + H_2O_2 + EGCG	0.5	3.4 ± 4.6	96.6 ± 4.6	0.5486
6	p + ^{NP}CuO + H_2O_2 + EGCG	1	0.7 ± 0.8	99.3 ± 0.8	0.0099
7	p + ^{NP}CuO + H_2O_2 + EGCG	2	0.0 ± 0.0	100.0 ± 0.0	0.32
8	p + ^{NP}CuO + H_2O_2 + EGCG	5	5.3 ± 5.4	94.7 ± 5.4	>0.9999
9	p + ^{NP}CuO + H_2O_2 + EGCG	10	18.5 ± 20.0	81.5 ± 20.0	0.3714
10	p + ^{NP}CuO + H_2O_2 + EGCG	25	33.0 ± 24.3	67.0 ± 24.3	0.187
11	p + ^{NP}CuO + H_2O_2 + EGCG	50	34.9 ± 22.6	65.1 ± 22.6	0.1514
12	p + ^{NP}CuO + H_2O_2 + EGCG	100	35.1 ± 20.1	64.9 ± 20.1	0.1241
13	p + ^{NP}CuO + H_2O_2 + EGCG	200	35.7 ± 18.9	64.3 ± 18.9	0.1083
14	p + ^{NP}CuO + H_2O_2 + EGCG	400	35.1 ± 15.7	64.9 ± 15.7	0.0814
15	p + ^{NP}CuO + H_2O_2 + EGCG	800	45.6 ± 17.8	54.4 ± 17.8	0.0593

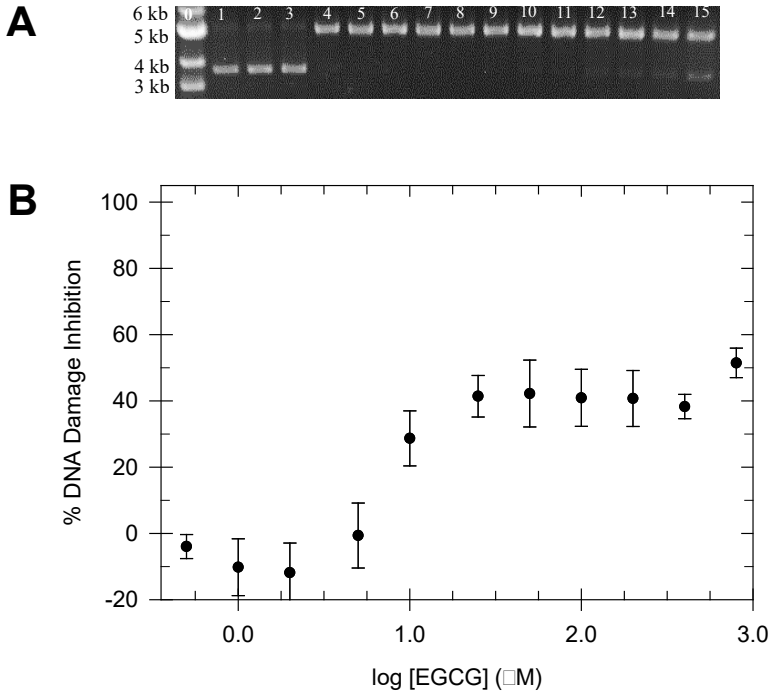

Figure S5. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and EGCG (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + EGCG (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of EGCG (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to EGCG concentration in the presence of ^{NP}CuO and H_2O_2.

Table S6. DNA damage inhibition by protocatechuic acid (PCA; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[PCA] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	96.6 ± 2.3	3.4 ± 2.3	-
2	p + H_2O_2 (50 μM)	0	98.2 ± 2.0	1.8 ± 2.0	-
3	p + PCA (800 μM)	800	89.3 ± 6.8	10.7 ± 6.8	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	23.8 ± 28.1	76.2 ± 28.1	-
5	p + ^{NP}CuO + H_2O_2 + PCA	0.5	17.5 ± 21.0	82.5 ± 21.0	0.6551
6	p + ^{NP}CuO + H_2O_2 + PCA	1	10.4 ± 12.3	89.6 ± 12.3	0.1998
7	p + ^{NP}CuO + H_2O_2 + PCA	2	7.9 ± 9.6	92.1 ± 9.6	0.1031
8	p + ^{NP}CuO + H_2O_2 + PCA	5	5.1 ± 8.7	94.9 ± 8.7	0.0652
9	p + ^{NP}CuO + H_2O_2 + PCA	10	10.9 ± 12.4	89.1 ± 12.4	0.2134
10	p + ^{NP}CuO + H_2O_2 + PCA	25	11.6 ± 14.7	88.4 ± 14.7	0.2871
11	p + ^{NP}CuO + H_2O_2 + PCA	50	11.7 ± 13.6	88.3 ± 13.6	0.2632
12	p + ^{NP}CuO + H_2O_2 + PCA	100	16.2 ± 18.9	83.8 ± 18.9	0.5582
13	p + ^{NP}CuO + H_2O_2 + PCA	200	15.7 ± 16.8	84.3 ± 16.8	0.4915
14	p + ^{NP}CuO + H_2O_2 + PCA	400	25.1 ± 19.8	74.9 ± 19.8	0.9198
15	p + ^{NP}CuO + H_2O_2 + PCA	800	35.3 ± 12.8	64.7 ± 12.8	0.26

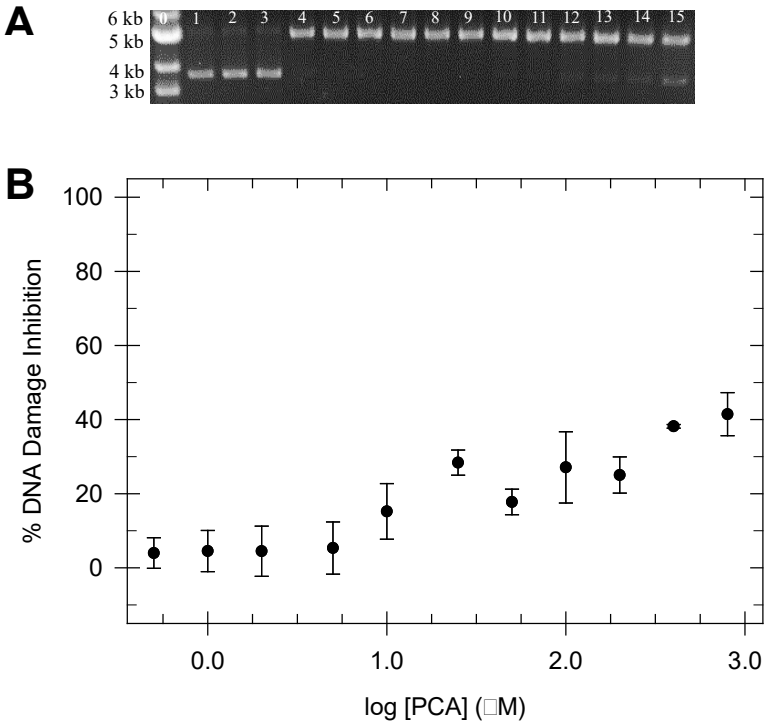

Figure S6. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and PCA (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + PCA (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of PCA (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to PCA concentration in the presence of ^{NP}CuO and H_2O_2.

Table S7. DNA damage inhibition by vanillic acid (VA; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[VA] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	93.7 ± 3.5	6.3 ± 3.5	-
2	p + H_2O_2 (50 μM)	0	93.2 ± 6.6	6.8 ± 6.6	-
3	p + VA (800 μM)	800	92.7 ± 7.4	7.3 ± 7.4	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	2.4 ± 4.0	97.6 ± 4.0	-
5	p + ^{NP}CuO + H_2O_2 + VA	0.5	0.0 ± 0.0	100.0 ± 0.0	0.0002
6	p + ^{NP}CuO + H_2O_2 + VA	1	0.2 ± 0.2	99.8 ± 0.2	0.0027
7	p + ^{NP}CuO + H_2O_2 + VA	2	0.0 ± 0.0	100.0 ± 0.0	0.0002
8	p + ^{NP}CuO + H_2O_2 + VA	5	0.2 ± 0.3	99.8 ± 0.3	0.0061
9	p + ^{NP}CuO + H_2O_2 + VA	10	3.1 ± 5.3	96.9 ± 5.3	0.8403
10	p + ^{NP}CuO + H_2O_2 + VA	25	1.2 ± 2.1	98.8 ± 2.1	0.4266
11	p + ^{NP}CuO + H_2O_2 + VA	50	0.1 ± 0.1	99.9 ± 0.1	0.0006
12	p + ^{NP}CuO + H_2O_2 + VA	100	0.1 ± 0.1	99.9 ± 0.1	0.0006
13	p + ^{NP}CuO + H_2O_2 + VA	200	0.0 ± 0.0	100.0 ± 0.0	0.0002
14	p + ^{NP}CuO + H_2O_2 + VA	400	0.1 ± 0.1	99.9 ± 0.1	0.0006
15	p + ^{NP}CuO + H_2O_2 + VA	800	0.1 ± 0.1	99.9 ± 0.1	0.0006

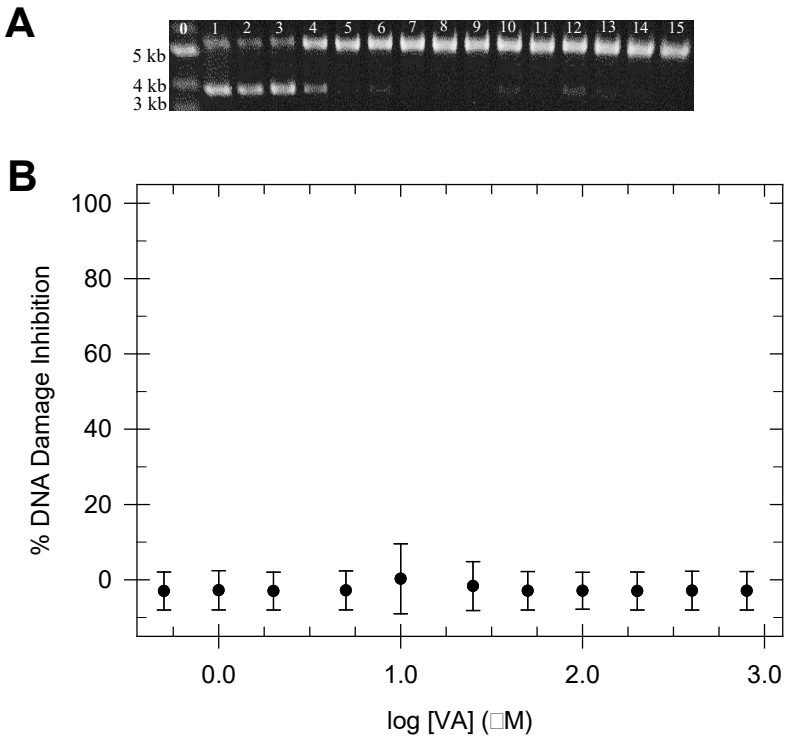

Figure S7. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and VA (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + VA (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of VA (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to VA concentration in the presence of ^{NP}CuO and H_2O_2.

Table S8. DNA damage inhibition by epicatechin (EC; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[EC] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	97.8 ± 0.5	2.2 ± 0.5	-
2	p + H_2O_2 (50 μM)	0	94.0 ± 3.9	6.0 ± 3.9	-
3	p + EC (800 μM)	800	95.4 ± 4.4	4.6 ± 4.4	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	2.4 ± 2.7	97.6 ± 2.7	-
5	p + ^{NP}CuO + H_2O_2 + EC	0.5	5.2 ± 8.4	94.8 ± 8.4	0.622
6	p + ^{NP}CuO + H_2O_2 + EC	1	0.2 ± 0.3	99.8 ± 0.3	0.0061
7	p + ^{NP}CuO + H_2O_2 + EC	2	1.0 ± 1.3	99.0 ± 1.3	0.2031
8	p + ^{NP}CuO + H_2O_2 + EC	5	0.4 ± 0.4	99.6 ± 0.4	0.0131
9	p + ^{NP}CuO + H_2O_2 + EC	10	1.1 ± 1.4	98.9 ± 1.4	0.249
10	p + ^{NP}CuO + H_2O_2 + EC	25	1.7 ± 1.5	98.3 ± 1.5	0.5038
11	p + ^{NP}CuO + H_2O_2 + EC	50	0.0 ± 0.0	100.0 ± 0.0	>0.9999
12	p + ^{NP}CuO + H_2O_2 + EC	100	0.0 ± 0.0	100.0 ± 0.0	>0.9999
13	p + ^{NP}CuO + H_2O_2 + EC	200	0.0 ± 0.0	100.0 ± 0.0	>0.9999
14	p + ^{NP}CuO + H_2O_2 + EC	400	0.0 ± 0.0	100.0 ± 0.0	>0.9999
15	p + ^{NP}CuO + H_2O_2 + EC	800	0.0 ± 0.0	100.0 ± 0.0	>0.9999

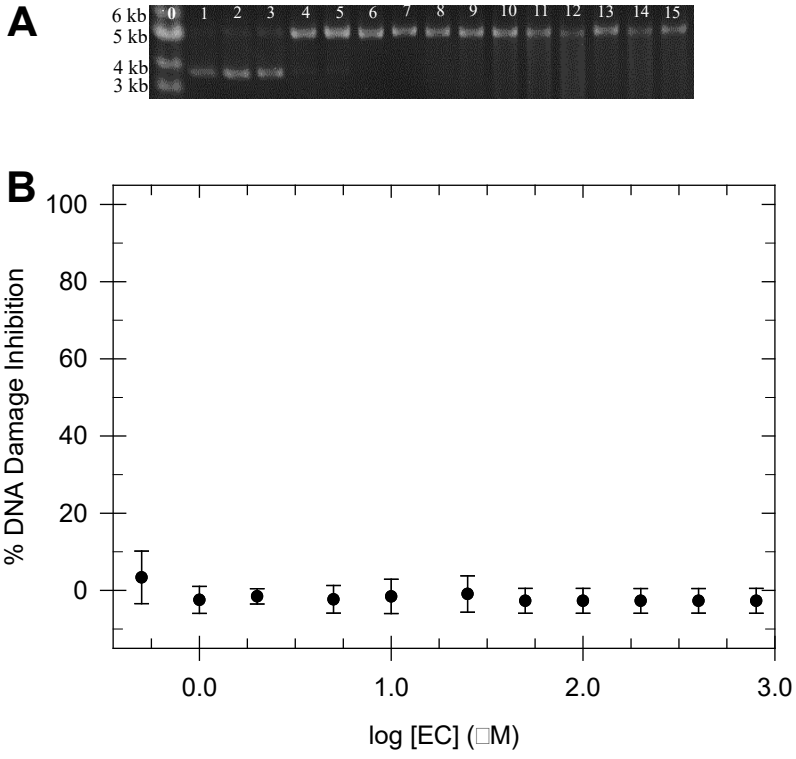

Figure S8. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and EC (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + EC (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of EC (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to EC concentration in the presence of ^{NP}CuO and H_2O_2.

Table S9. DNA damage inhibition by gallic acid (GA; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[GA] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid (p)	0	93.1 ± 1.1	6.9 ± 1.1	-
2	p + H_2O_2 (50 μM)	0	97.6 ± 0.5	2.4 ± 0.5	-
3	p + GA (800 μM)	800	94.8 ± 3.6	5.2 ± 3.6	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	8.7 ± 2.0	91.3 ± 2.0	-
5	p + ^{NP}CuO + H_2O_2 + GA	0.5	6.5 ± 5.9	93.5 ± 5.9	0.5846
6	p + ^{NP}CuO + H_2O_2 + GA	1	5.3 ± 8.7	94.7 ± 8.7	0.5683
7	p + ^{NP}CuO + H_2O_2 + GA	2	0.0 ± 0.0	100.0 ± 0.0	>0.9999
8	p + ^{NP}CuO + H_2O_2 + GA	5	0.0 ± 0.0	100.0 ± 0.0	>0.9999
9	p + ^{NP}CuO + H_2O_2 + GA	10	0.0 ± 0.0	100.0 ± 0.0	>0.9999
10	p + ^{NP}CuO + H_2O_2 + GA	25	0.0 ± 0.0	100.0 ± 0.0	>0.9999
11	p + ^{NP}CuO + H_2O_2 + GA	50	0.3 ± 0.5	99.7 ± 0.5	0.0012
12	p + ^{NP}CuO + H_2O_2 + GA	100	0.0 ± 0.0	100.0 ± 0.0	>0.9999
13	p + ^{NP}CuO + H_2O_2 + GA	200	0.0 ± 0.0	100.0 ± 0.0	>0.9999
14	p + ^{NP}CuO + H_2O_2 + GA	400	0.0 ± 0.0	100.0 ± 0.0	>0.9999
15	p + ^{NP}CuO + H_2O_2 + GA	800	0.0 ± 0.0	100.0 ± 0.0	>0.9999

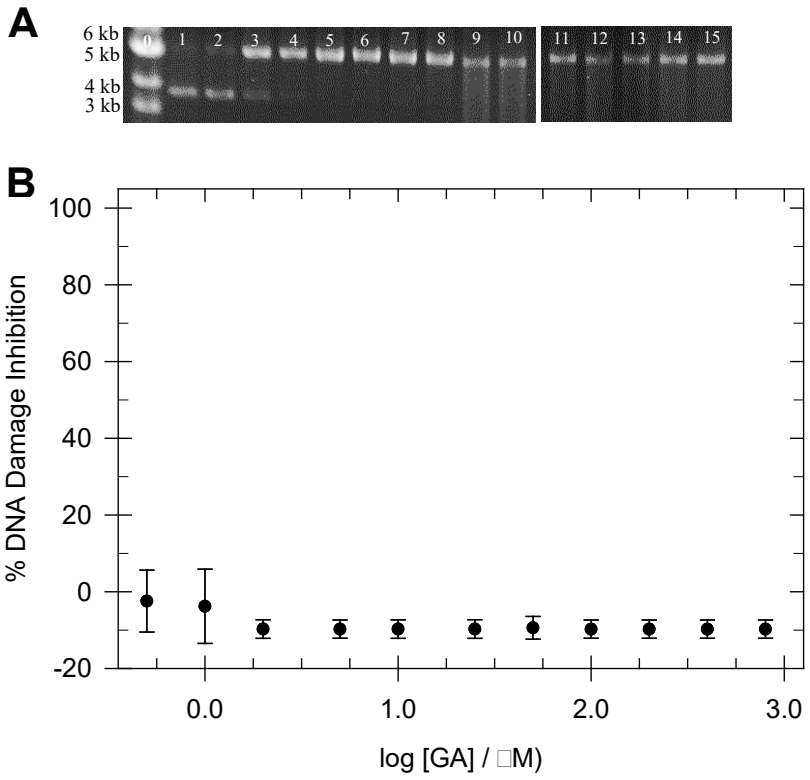

Figure S9. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and GA (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + GA (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of GA (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to GA concentration in the presence of ^{NP}CuO and H_2O_2.

Table S10. DNA damage inhibition by epigallocatechin (EGC; 0.5 – 800 μM) with ^{NP}CuO (500 μM) and H_2O_2 (50 μM) at pH 7 for 150 min.

Gel lane	Contents	[EGC] (μM)	% Supercoiled DNA	% Nicked DNA	*p* Value
1	plasmid + diH_2O	0	98.3 ± 1.6	1.7 ± 1.6	-
2	p + H_2O_2 (50 μM)	0	98.5 ± 0.4	1.5 ± 0.4	-
3	p + EGC (800 μM)	800	31.3 ± 5.1	68.7 ± 5.1	-
4	p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM)	0	0.5 ± 0.7	99.5 ± 0.7	-
5	p + ^{NP}CuO + H_2O_2 + EGC	0.5	0.0 ± 0.0	100.0 ± 0.0	>0.9999
6	p + ^{NP}CuO + H_2O_2 + EGC	1	0.0 ± 0.0	100.0 ± 0.0	>0.9999
7	p + ^{NP}CuO + H_2O_2 + EGC	2	0.0 ± 0.0	100.0 ± 0.0	>0.9999
8	p + ^{NP}CuO + H_2O_2 + EGC	5	0.7 ± 0.7	99.3 ± 0.7	0.6697
9	p + ^{NP}CuO + H_2O_2 + EGC	10	1.2 ± 0.8	98.8 ± 0.8	0.2689
10	p + ^{NP}CuO + H_2O_2 + EGC	25	0.0 ± 0.0	100.0 ± 0.0	>0.9999
11	p + ^{NP}CuO + H_2O_2 + EGC	50	0.0 ± 0.0	100.0 ± 0.0	>0.9999
12	p + ^{NP}CuO + H_2O_2 + EGC	100	0.0 ± 0.0	100.0 ± 0.0	>0.9999
13	p + ^{NP}CuO + H_2O_2 + EGC	200	0.0 ± 0.0	100.0 ± 0.0	>0.9999
14	p + ^{NP}CuO + H_2O_2 + EGC	400	0.0 ± 0.0	100.0 ± 0.0	>0.9999
15	p + ^{NP}CuO + H_2O_2 + EGC	800	0.0 ± 0.0	100.0 ± 0.0	>0.9999

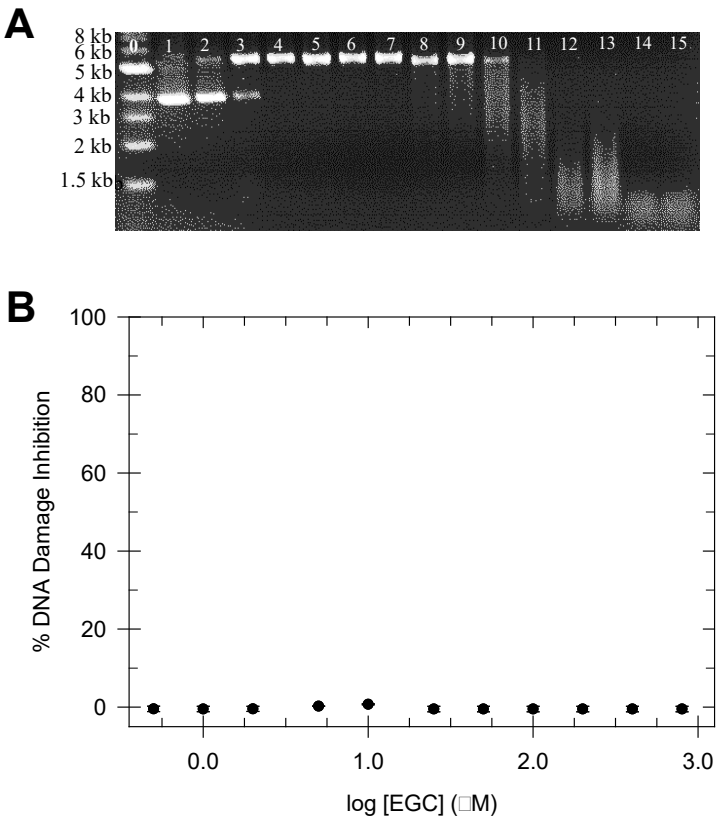

Figure S10. (A) Gel electrophoresis image of DNA treated with ^{NP}CuO (500 μM), H_2O_2 (50 μM), and EGC (0.5 – 800 μM) at pH 7 (MOPS buffer) for 150 min. Lane 0: 1 kb molecular weight ladder; lane 1: plasmid (p); lane 2: p + H_2O_2 (50 μM); lane 3: p + EGC (800 μM); lane 4: p + ^{NP}CuO (500 μM) + H_2O_2 (50 μM); lanes 5-15: p + ^{NP}CuO (500 μM) + increasing concentrations of EGC (0.5, 1, 2, 5, 10, 25, 50, 100, 200, 400, and 800 μM, respectively) and H_2O_2 (50 μM). (B) Percentage of DNA damage inhibition with respect to EGC concentration in the presence of ^{NP}CuO and H_2O_2.

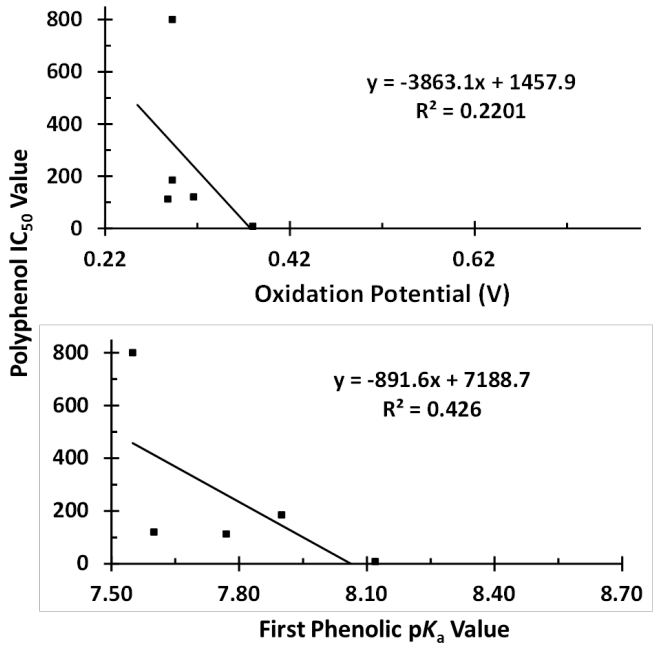

Figure S11. Relationship between the IC_{50} value for polyphenol prevention of $^{NP}CuO/H_2O_2$-mediated DNA damage and polyphenol oxidation potential (top) and pK_a of the most acidic phenolic hydrogen (bottom). The equation of the best-fit lines and their correlation coefficients are indicated on each graph.

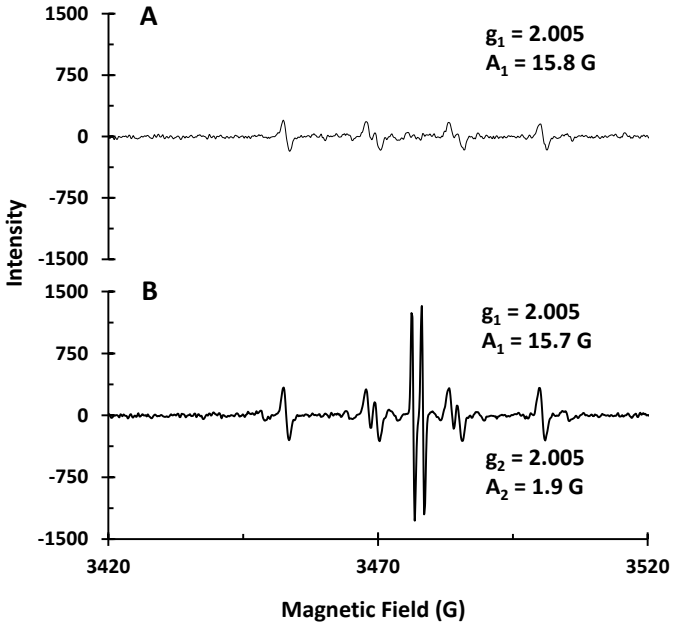

Figure S12. EPR spectra at room temperature of: (A) ^{NP}CuO (300 μM), H_2O_2 (22.5 mM), and (B) ^{NP}CuO (300 μM), H_2O_2 (22.5 mM), and ascorbic acid (375 μM). All spectra were collected at room temperature at pH 7 (MOPS 10 mM) with TEMP (30 mM) as spin trap. Parameters g_1 and A_1 correspond to TEMPO resonances, and parameters g_2 and A_2 correspond to ascorbyl radical resonances. Experimental conditions: time constant 81.92 ms, conversion time 81.92 ms, modulation amplitude 1.00 G, microwave power 20.02, and magnetic field 3500 ± 100 G.

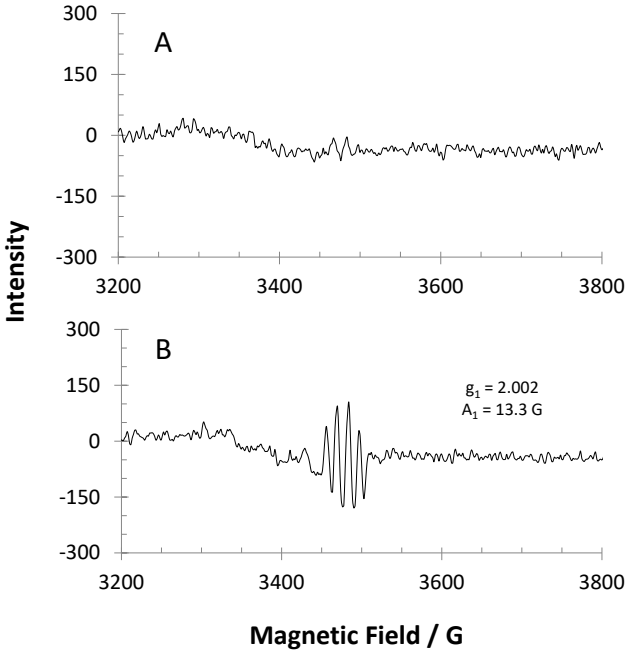

Figure S13. EPR spectra at room temperature of A) H_2O_2 (22.5 mM) and B) K_2O (5 mM). Spectra were collected at room temperature at pH 7 (MOPS 10 mM) with DMPO (30 mM) as spin trap. Parameters g_1 and A_1 correspond to DMPO-OH radical resonances. Experimental conditions: time constant 81.92 ms, conversion time 81.92 ms, modulation amplitude 1.00 G, microwave power 20.02, and magnetic field 3500 ± 300 G.

Table S11. Dynamic light scattering measurements of ^{NP}CuO and ^{w}CuO size, weighted by intensity.

CuO Mean Diameter (nm)		
Solvent	**H_2O**	**EMEM**
^{NP}CuO	175.1 ± 13.9	139.9 ± 1.8
^{w}CuO	186.3 ± 13.2	154.4 ± 11.1

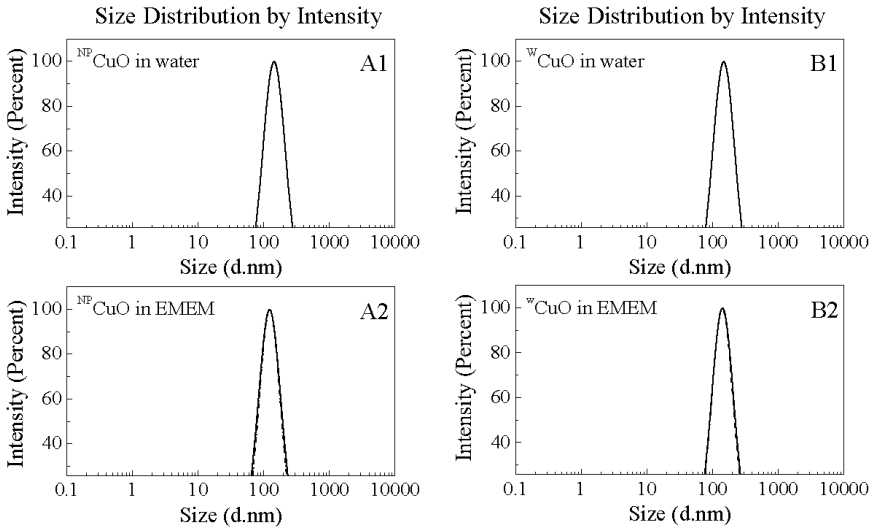

Figure S14. Size distribution by intensity of (A) ^{NP}CuO and (B) ^{w}CuO in water and EMEM, respectively.

Table S12. Effect of ^{NP}CuO (15.625 – 625 µM) on L929 cell viability after 24 h.

Contents	[^{NP}CuO] (µM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + ^{NP}CuO (15.625 µM)	15.625	91.8 ± 4.1	8.2 ± 4.1
L929 cells + ^{NP}CuO	31.25	97.7 ± 3.4	2.3 ± 3.4
L929 cells + ^{NP}CuO	62.5	62.0 ± 9.0	38.0 ± 9.0
L929 cells + ^{NP}CuO	156.25	33.7 ± 2.6	66.3 ± 2.6
L929 cells + ^{NP}CuO	312.5	0.2 ± 0.2	99.8 ± 0.2
L929 cells + ^{NP}CuO	625	1.0 ± 0.3	99.0 ± 0.3

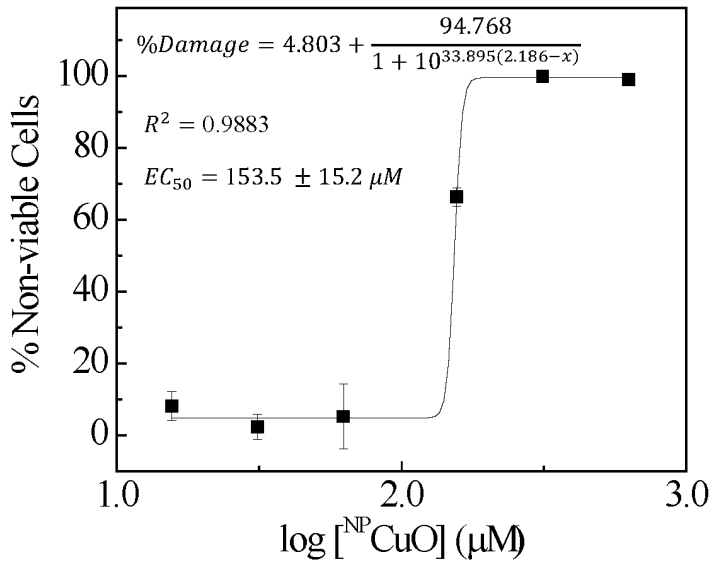

Figure S15. Graph of L929 cell viability with respect to ^{NP}CuO concentration showing the best-fit, sigmoidal EC_{50} curve.

Table S13. Effect of washed CuO (^{w}CuO 15.625 – 625 µM) on L929 cell viability after 24 h.

Contents	[^{w}CuO] (µM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + ^{w}CuO (15.625 µM)	15.625	81.6 ± 3.2	18.4 ± 3.2
L929 cells + ^{w}CuO	31.25	76.0 ± 9.0	24.0 ± 9.0
L929 cells + ^{w}CuO	62.5	83.9 ± 7.5	16.1 ± 7.5
L929 cells + ^{w}CuO	156.25	58.9 ± 7.0	41.1 ± 7.0
L929 cells + ^{w}CuO	312.5	28.4 ± 3.7	71.6 ± 3.7
L929 cells + ^{w}CuO	625	25.1 ± 8.2	74.9 ± 8.2

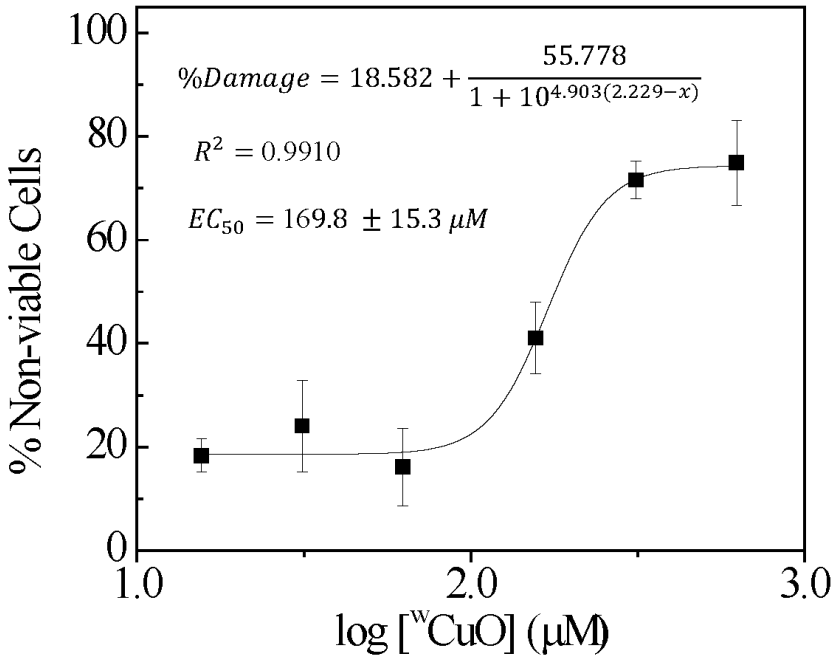

Figure S16. Graph of L929 cell viability with respect to ^{w}CuO concentration showing the best-fit, sigmoidal EC_{50} curve.

Table S14. Effect of dissolved copper (as $CuCl_2$; 0.165 – 6.6 µM) on L929 cell viability after 24 h.

Contents	$[Cu^{2+}]$ (µM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + Cu^{2+} (0.165 µM)	0.165	94.5 ± 14.0	5.5 ± 14.0
L929 cells + Cu^{2+}	0.33	96.2 ± 8.3	3.8 ± 8.3
L929 cells + Cu^{2+}	0.66	99.8 ± 2.2	0.2 ± 2.2
L929 cells + Cu^{2+}	1.65	98.7 ± 9.9	1.3 ± 9.9
L929 cells + Cu^{2+}	3.3	71.6 ± 2.8	28.4 ± 2.8
L929 cells + Cu^{2+}	6.6	73.0 ± 8.6	27.0 ± 8.6

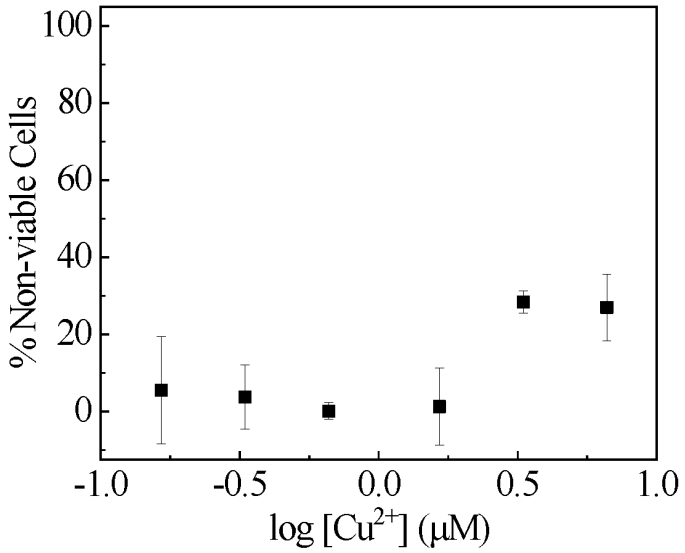

Figure S17. Graph of L929 cell viability with respect to dissolved copper concentration.

Table S15. Effect of CuO nanoparticle leachate after removal of dissolved Cu ions ([l]CuO; 0.6 – 12.0 mM) on L929 cell viability after 24 h.

Contents	[[l]CuO] (mM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + [l]CuO (0.6 mM)	0.6	89.1 ± 0.3	10.9 ± 0.3
L929 cells + [l]CuO	1.2	85.7 ± 2.1	14.3 ± 2.1
L929 cells + [l]CuO	1.5	69.4 ± 8.6	30.6 ± 8.6
L929 cells + [l]CuO	2.4	84.4 ± 6.2	15.6 ± 6.2
L929 cells + [l]CuO	3.0	90.4 ± 5.7	9.6 ± 5.7
L929 cells + [l]CuO	4.8	85.5 ± 5.1	14.5 ± 5.1
L929 cells + [l]CuO	6.0	79.4 ± 1.6	20.6 ± 1.6
L929 cells + [l]CuO	9.0	89.2 ± 0.3	10.8 ± 0.3
L929 cells + [l]CuO	12.0	73.4 ± 5.2	26.6 ± 5.2

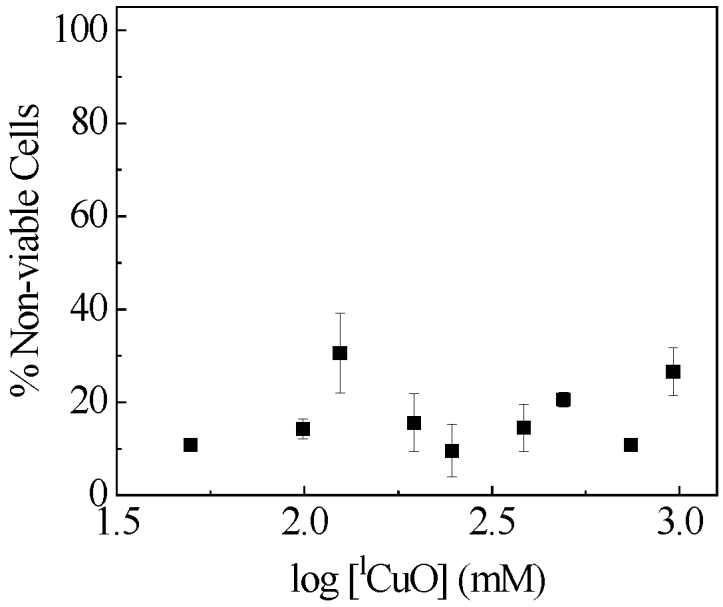

Figure S18. Graph of L929 cell viability with respect to CuO nanoparticle leachate concentration.

Table S16. Effect of H_2O_2 (50 - 2500 µM) on L929 cell viability after 24 h.

Contents	[H_2O_2] (µM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + H_2O_2 (50 µM)	50	93.4 ± 3.7	6.6 ± 3.7
L929 cells + H_2O_2	100	83.7 ± 12.3	16.3 ± 12.3
L929 cells + H_2O_2	200	17.9 ± 17.9	82.1 ± 17.9
L929 cells + H_2O_2	400	9.2 ± 8.0	90.8 ± 8.0
L929 cells + H_2O_2	500	7.3 ± 7.1	92.7 ± 7.1
L929 cells + H_2O_2	1000	5.5 ± 2.5	94.5 ± 2.5
L929 cells + H_2O_2	2000	5.1 ± 4.8	94.9 ± 4.8
L929 cells + H_2O_2	2500	0.7 ± 0.8	99.3 ± 0.8

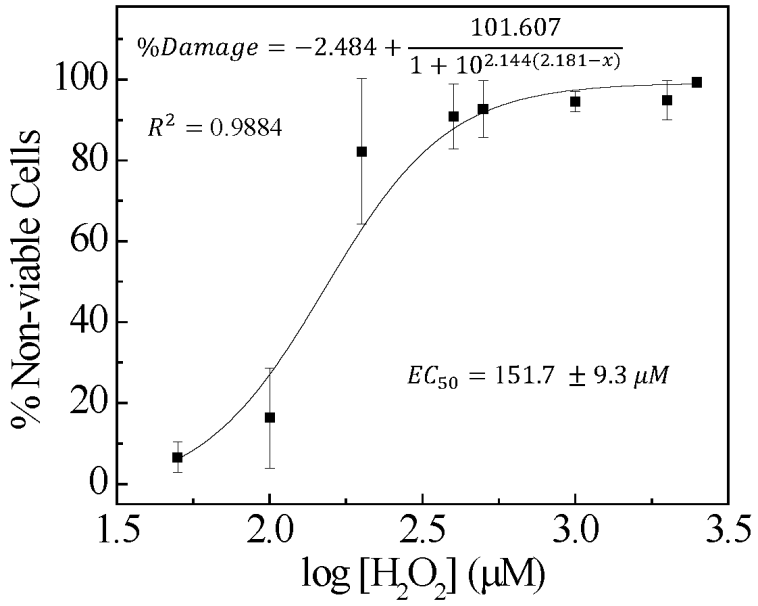

Figure S19. Graph of L929 cell viability with respect to H_2O_2 concentration showing the best-fit, sigmoidal EC_{50} curve.

Table S17. Effect of ^{NP}CuO (62.5 μM) and H_2O_2 (50 - 2500 μM) on L929 cell viability after 24 h.

Contents	[H_2O_2] (μM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + H_2O_2 (50 μM)	50	93.4 ± 3.7	6.6 ± 3.7
L929 cells + ^{NP}CuO (62.5 μM)	0	94.7 ± 9.0	5.3 ± 9.0
L929 cells + ^{NP}CuO + H_2O_2 (50 μM)	50	12.9 ± 9.4	87.1 ± 9.4
L929 cells + ^{NP}CuO + H_2O_2	100	11.3 ± 4.7	88.7 ± 4.7
L929 cells + ^{NP}CuO + H_2O_2	200	3.0 ± 1.6	97.0 ± 1.6
L929 cells + ^{NP}CuO + H_2O_2	400	3.7 ± 1.6	96.3 ± 1.6
L929 cells + ^{NP}CuO + H_2O_2	500	0.3 ± 0.4	99.7 ± 0.4
L929 cells + ^{NP}CuO + H_2O_2	1000	1.7 ± 2.6	98.3 ± 2.6
L929 cells + ^{NP}CuO + H_2O_2	2000	2.1 ± 0.6	97.9 ± 0.6
L929 cells + ^{NP}CuO + H_2O_2	2500	2.2 ± 0.9	97.8 ± 0.9

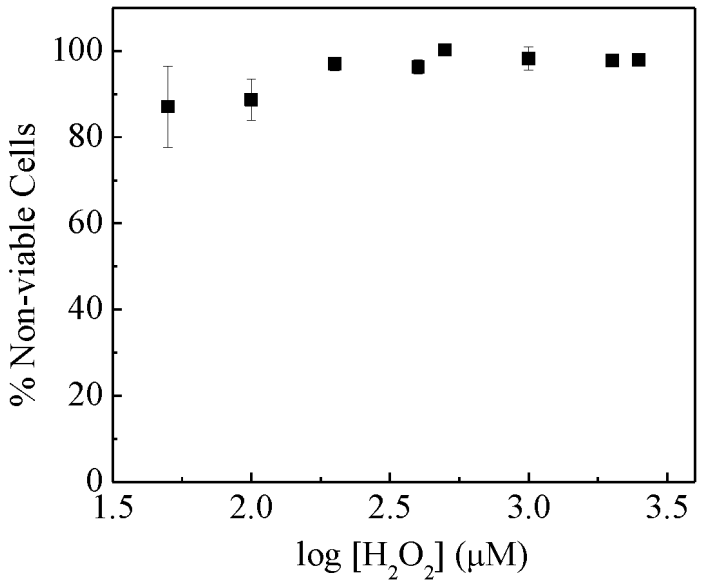

Figure S20. Graph of L929 cell viability with respect to H_2O_2 concentration in the presence of ^{NP}CuO/H_2O_2.

Table S18. Effect of methyl-3,4-dihydroxybenzoate (MEPCA; 1.0 – 400 µM) with ^{NP}CuO (153.5 µM) and H_2O_2 (80 µM) on L929 cell viability after 24 h.

Contents	[MEPCA] (µM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + H_2O_2 (80 µM)	0	53.9 ± 5.8	46.1 ± 5.8
L929 cells + MEPCA (400 µM)	400	67.0 ± 12.7	33.0 ± 12.7
L929 cells + ^{NP}CuO (153.5 µM) + H_2O_2 (80 µM)	0	17.3 ± 0.6	82.7 ± 0.6
L929 cells + MEPCA + ^{NP}CuO + H_2O_2	1	80.6 ± 7.4	19.4 ± 7.4
L929 cells + MEPCA + ^{NP}CuO + H_2O_2	10	61.7 ± 4.8	38.3 ± 4.8
L929 cells + MEPCA + ^{NP}CuO + H_2O_2	50	31.7 ± 4.2	68.3 ± 4.2
L929 cells + MEPCA + ^{NP}CuO + H_2O_2	100	26.2 ± 8.7	73.8 ± 8.7
L929 cells + MEPCA + ^{NP}CuO + H_2O_2	200	17.9 ± 5.0	82.1 ± 5.0
L929 cells + MEPCA + ^{NP}CuO + H_2O_2	400	13.7 ± 0.3	86.3 ± 0.3

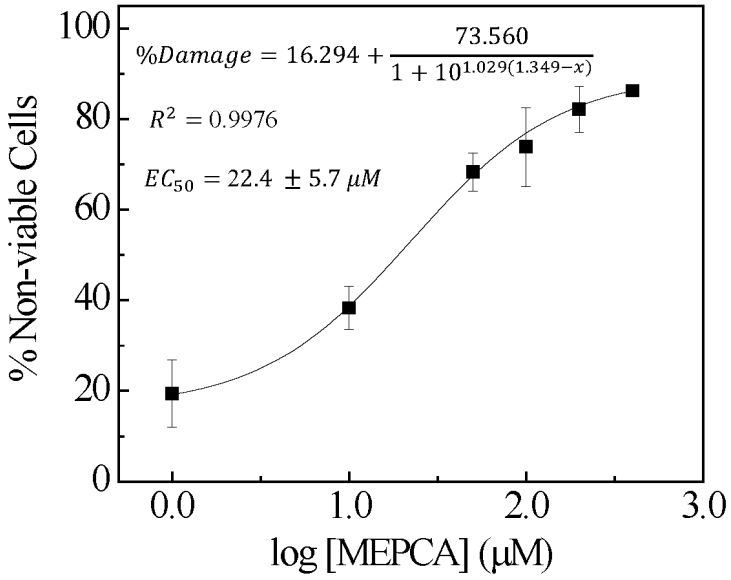

Figure S21. Graph of L929 cell viability with respect to MEPCA concentration in the presence of ^{NP}CuO/H_2O_2.

Table S19. L929 cell damage inhibition by methyl-3,4,5-trihydroxybenzoate (MEGA; 1.0 – 400.0 μM) with ^{NP}CuO (153.5 μM) and H_2O_2 (80 μM) after 24 h.

Contents	[MEGA] (μM)	% Viable Cells	% Non-viable Cells
L929 cells	0	100.0 ± 0.0	0.0 ± 0.0
L929 cells + H_2O_2 (80 μM)	0	53.9 ± 5.8	46.1 ± 5.8
L929 cells + MEGA (400 μM)	400	39.7 ± 12.6	60.3 ± 12.6
L929 cells + ^{NP}CuO (153.5 μM) + H_2O_2 (80 μM)	0	17.3 ± 0.6	82.7 ± 0.6
L929 cells + MEGA + ^{NP}CuO + H_2O_2	1	70.6 ± 7.5	29.4 ± 7.5
L929 cells + MEGA + ^{NP}CuO + H_2O_2	10	62.4 ± 0.5	37.6 ± 0.5
L929 cells + MEGA + ^{NP}CuO + H_2O_2	50	33.0 ± 7.5	67.0 ± 7.5
L929 cells + MEGA + ^{NP}CuO + H_2O_2	100	38.3 ± 6.3	61.7 ± 6.3
L929 cells + MEGA + ^{NP}CuO + H_2O_2	200	34.7 ± 14.5	65.3 ± 14.5
L929 cells + MEGA + ^{NP}CuO + H_2O_2	400	34.5 ± 10.9	65.5 ± 10.9

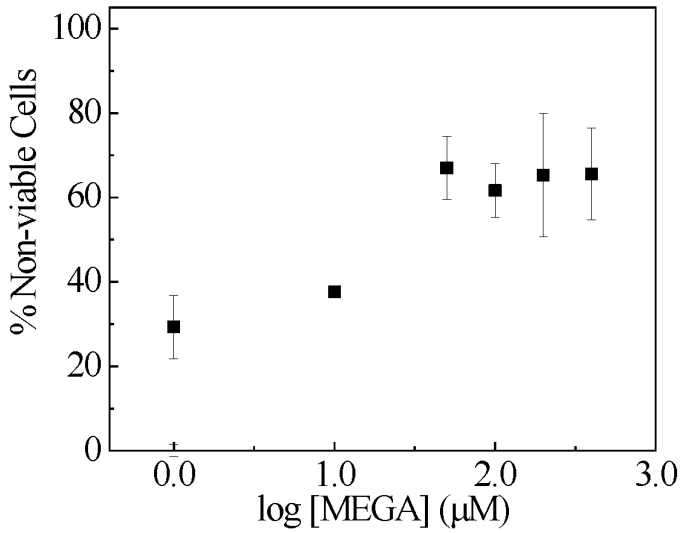

Figure S22. Graph of L929 cell viability with respect to MEGA concentration in the presence of ^{NP}CuO/H_2O_2.