Supplementary Information

Photochemical detoxification of Cr (VI) using iron and saccharic acid: Insights from cytotoxic and genotoxic assays

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2. Materials and Methods

2.1 Chemicals:

Iron(III) sulphate hydrate, Citric acid, Oxalic acid and Potassium dichromate were purchased from Merck. 1, 10 Phenanthroline, Potassium Saccharate and Diphenylcarbazide were purchased from Sigma-Aldrich. Humic acid, Sodium acetate trihydrate, Sodium chloride, Sodium sulfate, Sodium hydrogen carbonate, Sodium hydroxide, and Humic acid were purchased from S.D Fine Chemicals Ltd., India. All the chemicals used were of guaranteed analytical grade. All the solutions, buffers and aqueous reagents were freshly prepared with deionized water (resistivity 18.21 MΩ.cm) from the Millipore system.

2.3 Cyclic voltammetry:

Electrochemical experiments were performed with potentiostat PG16125 (Techno Science Instruments) in a three-electrode system including a saturated calomel electrode (SCE), a platinum counter electrode and a glassy carbon working electrode. The glassy carbon electrode was polished well using alumina slurry (0.3 and 0.03 μM), rinsed with isopropanol and distilled water followed by a sonication for a minute. 5 mM KCl was used as a supporting electrolyte. Before each scan, the sample solutions were purged with argon for 30 min to remove dissolved oxygen. Voltagrams were recorded at a scan rate of 5 mV s^{-1}.

2.4 Cell culture and MTT assay:

HeLa cells were obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured according to standard protocols (Hou et al. 2017, Sundaresan et al. 2008, Wang et al. 2016b). Briefly, the cells were grown on standard tissue culture polystyrene dishes using a regular high glucose Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotic- antimycotic mix. The cells were sub-cultured regularly at 80% confluence. For MTT assay the cells were seeded in 96 well plates and the cells were allowed to attach for 24 hrs. The cells were then incubated for 24 hours with (i)

Cr(VI) 200 µM (ii) Cr(VI) 200 µM treated with Fe(III)(50 µM) /UVC system for 30 min and (iii) Cr(VI) 200 µM treated with Fe(III)(50 µM) /SA (300 µM)/UVC system for 30 min. The cells were then incubated with 0.5 mg/l MTT (Sigma) dissolved in DMEM for 2 hrs. The resulting blue precipitate was dissolved in DMSO and the absorbance at 570 nm wavelength was recorded using a standard 96 well plate reader (Tecan). The % cell viability was calculated relative to the control cells. The data are represented as Mean ± SD from 3 independent samples, $p < 0.01$.

2.5 Confocal microscopy:

Confocal microscopy was carried out using standard procedures as described in our previous work (Jain et al. 2017). Briefly, HeLa cells were grown on sterilized glass cover slips and the above mentioned treatments were carried out 24 h after seeding. Post treatment, the cells were washed twice with Phosphate buffered saline (PBS) and fixed with 4 % formaldehyde for 15 min. The cells were then permeabilized with 0.2% Triton X-100 for 5 min. Blocking was carried out using a blocking solution (5 % BSA dissolved in PBS) for 1 h at room temperature. The cells were then incubated overnight at 4^{o} C with γ-H2AX (Cell signalling 9718) antibody dissolved in blocking solution at a dilution of 1:300. The cells were then washed with PBS and incubated with secondary antibody (Rabbit secondary conjugated with Alexa Fluor 546 fluorophore, Thermo) at a dilution of 1:500 in blocking solution for 1 hr at room temperature. Finally, the cells were washed with PBS and the nuclei were stained with Hoechst 33342 and were mounted on clean glass slides using the DPX mountant solution. The images were acquired using the LSM 710 confocal microscope and images were processed out using the Zen Lite software. The pie chart representing the percentage of nuclei displaying γH2AX foci was realized from the image.

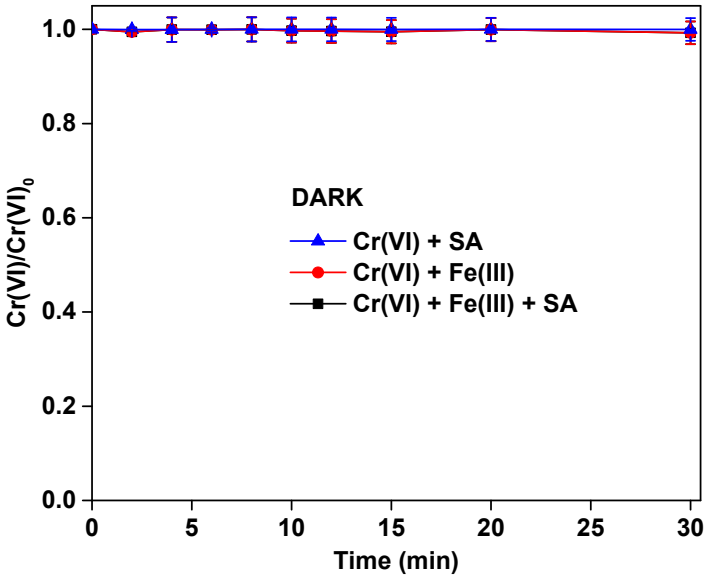

Fig. S1. Reduction of Cr(VI) as function of time by SA, Fe(III), and Fe(III)/SA in dark [Fe(III)] = 50 μM; [Cr(VI)] = 200 μM; [SA] = 300 μM; pH 3.

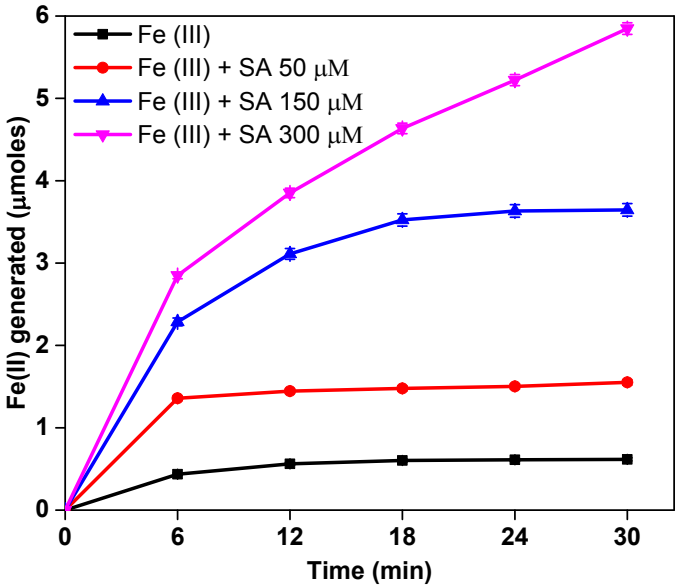

Fig. S2. Moles of Fe(II) generated upon photolysis of Fe(III) in presence of increasing concentration of SA. [Fe(III)] = 50 μM. Irradiance@254 nm = 0.33 mW cm^{-2}

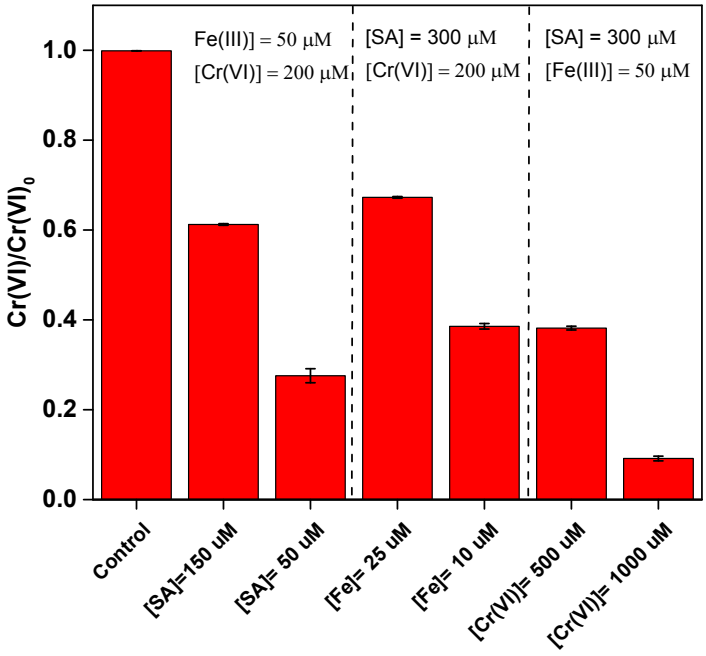

Fig. S3. Effect of initial concentration of Fe(III), SA and Cr (VI) on Cr(VI) photoreduction by Fe(III)/SA/UVC system. Treatment time – 30 Irradiance@254 nm = 0.33 mW cm^{-2}

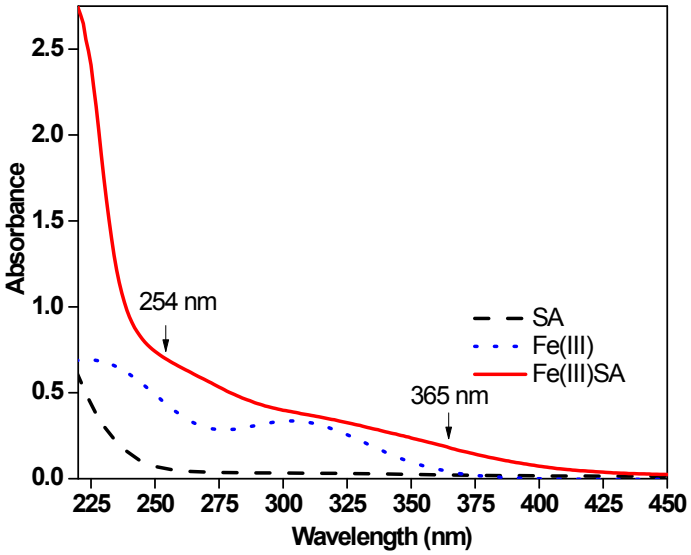

Fig. S4. UV-Visible absorption spectra of Fe(III) (150 µM), SA (2.5 mM) and Fe(III)SA (150 µM).

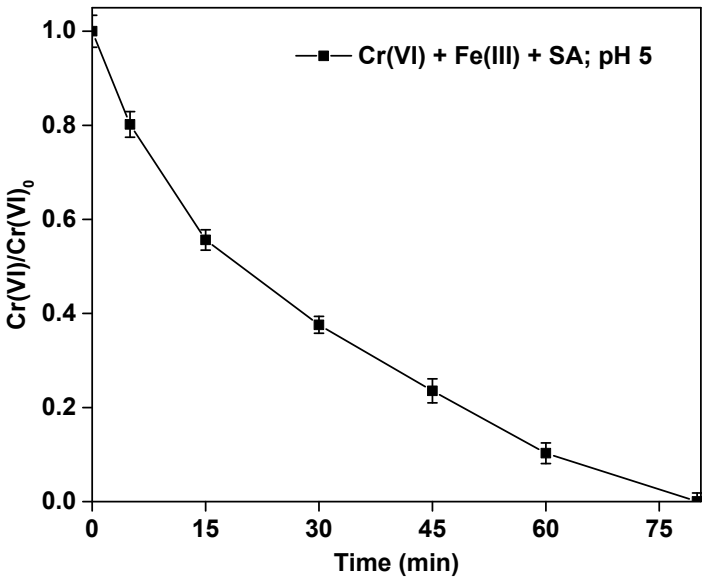

Fig. S5. Reduction of Cr(VI) as function of time by Fe(III)/SA/UVC system at pH 5. [Fe(III)] = 50 μM; [Cr(VI)] = 200 μM; [SA] = 300 μM; pH 3; Irradiance at 254 nm = 0.33 mW cm^{-2}

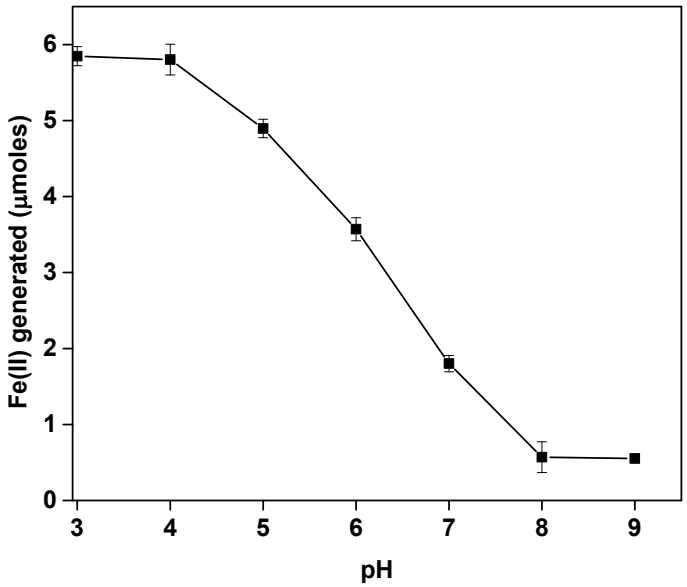

Fig. S6. Moles of Fe(II) generated upon photolysis of Fe(III) in presence of SA as function of pH. [Fe(III)] = 50 μM; [SA] = 300 μM; Irradiance@254 nm = 0.33 mW cm^{-2}

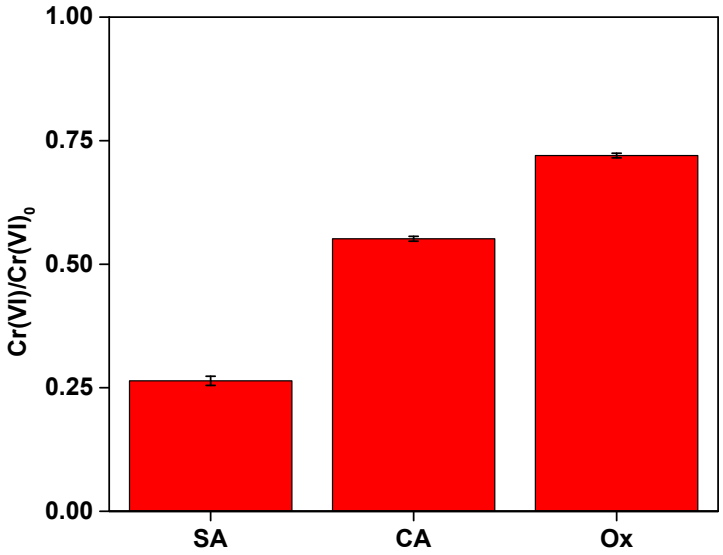

Fig. S7. Reduction of Cr(VI) by Fe(III)/SA/UVC, Fe(III)/CA/UVC and Fe(III)/Ox/UVC system. Treatment time = 15 min. [Fe(III)] = 50 μM; [Cr(VI)] = 200 μM; [L] = 300 μM; pH 3; Irradiance at 254 nm = 0.33 mW cm^{-2}

Table S 1. Water quality parameters of raw water samples:

Parameters	Method	Ground Water	Sankey Lake Sample
Chemical Oxygen Demand (C.O.D)	IS:3025(P-58)2006	4.5 ppm	76.8 ppm
Biological oxygen demand (BOD)	IS:3025(P-44)1993	Below Detection limit	26 ppm
Total Organic Carbon (TOC)	ISO 8245:1999(E)	1.7 ppm	20.5 ppm
pH	pH meter	6.1 ± 0.1	7.4 ± 0.3
Sulphate, as SO4	IS:3025 & APHA	11.92 ppm	19.56 ppm
Nitrate (as NO3)	IS:3025 & APHA	4.20 ppm	15.53 ppm
Chloride (as Cl)	IS:3025 & APHA	61.11 ppm	63.05 ppm
Phosphate (as PO_4)	IS:3025 (P-31) 2009	2 ppm	2.02 ppm
Total Hardness (as CaCO3)	IS:3025 (P-21) 2009	141.25 ppm	167.13 ppm
Total Dissolved Solids (mg/L)	IS:3025(P-16)-1984	336 ppm	586 ppm
Total Suspended Solids	IS:3025 (P-17) 1984	Absent	39 ppm

Table S 2. Composition of simulated galvanic waste water:

Parameters	Values
Chemical Oxygen Demand (C.O.D)	6.2 ppm
Biological oxygen demand (BOD)	Below detection limit
Total Organic Carbon (TOC)	3.5 ppm
Total Chromium	10 mg L^{-1}
Humic Acid	3 ppm
Sodium	90 mg L^{-1}
Magnesium	10 mg L^{-1}
Potassium	66 mg L^{-1}
Fluoride	1 mg L^{-1}
Chloride	30 mg L^{-1}
Nitrite	2 mg L^{-1}
Nitrate	25 L^{-1}
Zinc	5 mg L^{-1}
Aluminium	5 mg L^{-1}
Nickel	5 mg L^{-1}
Copper	5 mg L^{-1}

References:

1. Hou, C., Lu, G., Zhao, L., Yin, P. and Zhu, L. (2017) Estrogenicity assessment of membrane concentrates from landfill leachate treated by the UV-Fenton process using a human breast carcinoma cell line. Chemosphere 180, 192-200.

2. Jain, A., Ravi, V., Muhamed, J., Chatterjee, K. and Sundaresan, N.R. (2017) A simplified protocol for culture of murine neonatal cardiomyocytes on nanoscale keratin coated surfaces. International journal of cardiology 232, 160-170.

3. Sundaresan, N.R., Samant, S.A., Pillai, V.B., Rajamohan, S.B. and Gupta, M.P. (2008) SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. Molecular and cellular biology 28(20), 6384-6401.

4. Wang, G., Lu, G., Yin, P., Zhao, L. and Yu, Q.J. (2016b) Genotoxicity assessment of membrane concentrates of landfill leachate treated with Fenton reagent and UV-Fenton reagent using human hepatoma cell line. Journal of hazardous materials 307, 154-162.