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Supporting information for article:

Enhancement of radiosensitivity of oral carcinoma cells by Iodinated chlorin *p*6 copper complex in combination with synchrotron X-ray radiation

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S1. Xylenol orange Fricke Dosimetry

Fricke Xylenol orange (FX) solution was freshly prepared by dissolving ferrous ammonium sulphate (s d fine-chem ltd., India) in 50 mM sulphuric acid at a final concentration of 1 mM. To this, Xylenol orange (Himedia, India) was added at a final concentration of 0.1 mM. For X-ray dosimetry, 300 µl of FX solution was added to each well in a 96 well plate. For each X-ray dose, six wells (replicates) in a row were used. The multiwell plate containing FX solution in six alternate rows was exposed to varying doses of X-ray as described in materials and methods. After irradiation, the absorbance of FX solution at 560 nm was measured using a microplate reader. All the measurements were done within 30 – 60 min after irradiation.

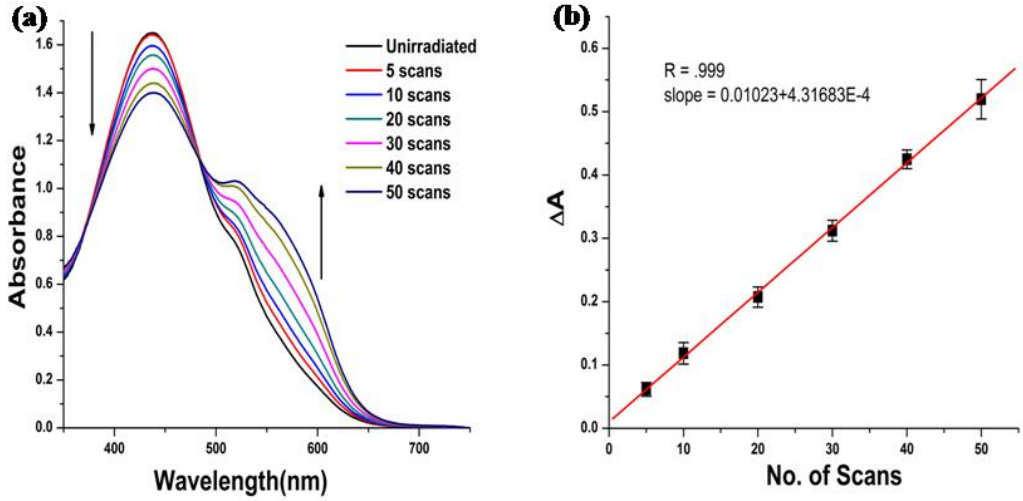

Figure S1 The absorbance spectra for irradiated samples of Xylenol orange Fricke solution (a). The dose response plot of change in absorbance at 560 nm (ΔA) of Xylenol orange Fricke solution vs No. of scans. Data represents mean ± SD obtained from three independent experiments.

Figure S1, shows the absorption spectra of irradiated Fricke Xylenol orange (FX) solution vs no. of scans. (5, 10, 20, 30, 40, 50). FX solution presents two absorption bands : one in the range from 435 -445 nm corresponding to Fe^{+2} ions initially present in non irradiated FX solution and another in the range of 550- 560 nm, corresponding to Fe^{+3} ions generated by radiation induced Fe^{+2} ions oxidation. With increase in dose delivered, the 435-445 nm band tends to decrease with concomitant increase in 550-560 nm band. Absorbed dose in the dosimetric solution is as follows:

$$D_w = \frac{(1.004 \times \Delta A)}{(\varepsilon\, G\, \rho\, d)} \quad (1)$$

where, D_w = absorbed dose in FX solution (Gy)

ΔA = net change in absorbance at 560 nm

ε = molar extinction coefficient at 560 nm (2000 m^2 mol^{-1})

ρ = density of FX solution (1024 Kg/m^3)

d = optical path length of FX solution (0.01 m)

G = radiochemical chemical yield (1.37 x 10^{-6} mol J^{-1} at an photon energy of 10 keV), according to International Commission on Radiation Units and Measurements (ICRU). The value of ε G was corrected for temperature and substituted in the equation (1)

$$\varepsilon_t G_t = \varepsilon G\,[\,1 + 0.007\,(t - 25)]\,\times [1 + 0.0015\,(t' - 25)] \quad (2)$$

Where, t = temperature (^{0}C) of FX solution during spectrophotometric measurement.

t' = temperature (^{0}C) of FX solution during irradiation which varied between 25 ± 3 ^{0}C.

Following the above equations a graph of no. of scan vs ΔA was plotted (Fig. S1 b). The slope of the straight line gives ΔA per scan which was calculated to be 33.5 cGy/scan.

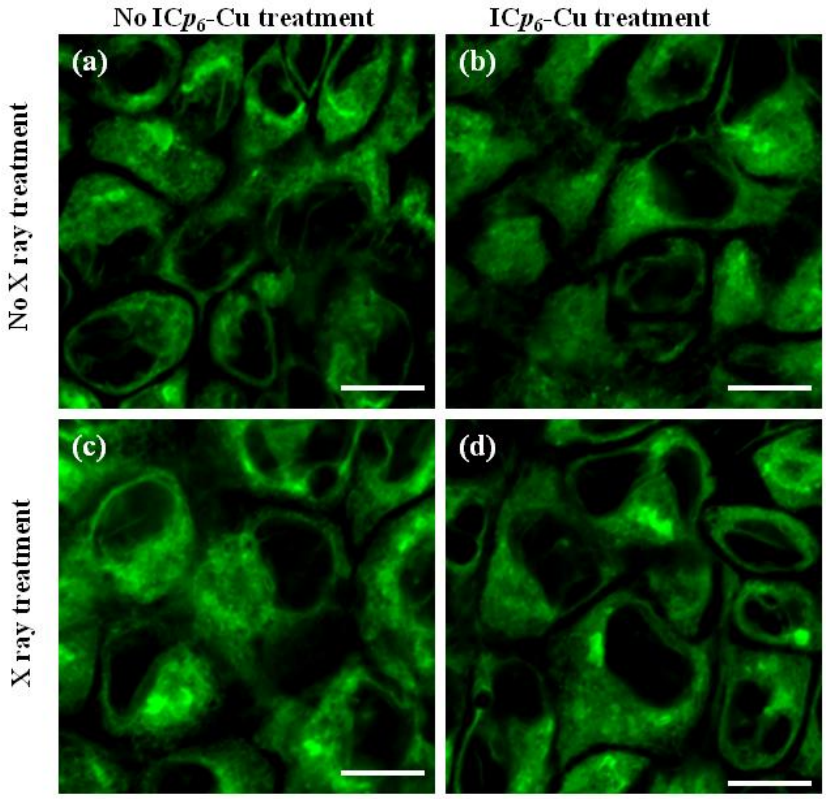

Figure S2 Confocal fluorescence micrographs of NT8e cells showing effect of ICp_6-Cu induced radiosensitization on integrity of ER. Cells were stained with Endopalsmic reticulum (ER) specific ER tracker probe. (a) Control, (b) ICp_6-Cu (30 μM) treatment alone, (c) X-ray (7 Gy) treatment alone and (d) ICp_6-Cu (30 μM) plus X-ray (7 Gy) treatment. Experiments were repeated three times with similar results and representative images are shown. Magnification 40X, scale bar 20 μm.