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Cellular evaluation of Diselenonicotinamide (DSNA) as a radioprotector against cell death and DNA damage

M. Raghuraman,[a,b,#] Prachi Verma,[b,c,#] Amit Kunwar,[b,c,*] Prasad P. Phadnis,[d] V.K. Jain,[d] K. Indira Priyadarsini[c,d,*]

[a] Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India-576104

[b] Radiation & Photochemistry Division, [c]Homi Bhabha National Institute, and [d]Chemistry Division, Bhabha Atomic Research Centre, Mumbai, India – 400094

# - Contributed equally to the research work

*Corresponding author
Radiation and Photochemistry Division,
Bhabha Atomic Research Centre,
Mumbai - 400085, India.
E-mail- kamit@barc.gov.in; kindira@barc.gov.in
Tel: 91-22-25592352/25595399, Fax: 91-22-25505151.

Supplementary figure legends

Figure S1. The cytotoxic effect of DSNA in CHO cells as determined by the clonogenic assay. The cells were treated with the increasing concentrations of DSNA for 24 h, washed with phosphate buffered saline (pH 7.4), supplemented with serum free DMEM medium and cultured for 7 days for the development of macroscopic colonies. (A) Plot of survival fraction against the concentration of DSNA. (B) Representative photographs showing colonies of CHO cells under different treatment conditions. About 250 cells were seeded for both control and DSNA treated groups. *$p < 0.05$ as compared to the control. Results are presented as mean ± SEM, n = 3.

Figure S2. Effect of DSNA pre-treatment (25 μM for 16 h) on the radiation (5 Gy) response of MCF7 cells as estimated by clonogenic assay. The inset shows the representative photographs of the colonies of MCF7 cells under different treatment conditions. About 250 cells for control groups and 750 cells for irradiated groups were seeded for this study. Results are presented as mean ± SEM, n = 3.

Figure S3. Effect of the pre-treatment (1-25 μM for 16 h) with diphenyl diselenide (Ph_2Se_2) on the radiation response of CHO cells as estimated by clonogenic assay. Representative photographs of the colonies of CHO cells under different treatment conditions are also shown. The results are presented as mean ± SEM, n = 3. CN – Control, IR – radiation.

Figure S4. Effects of DSNA treatment (25 μM) for 6 h and 48 h on the intracellular levels of GSH and GSSG in CHO cells. Results are presented as mean ± SEM, n = 3. *$p < 0.05$ as compared to the control.

Figure S5. Reduction of DSNA by NADPH catalyzed by rat liver TrxR. (A) DSNA (10-25 μM) in 0.2 ml of 50 mM Tris-HCl, 1 mM EDTA, pH 7.4 containing 100 μM NADPH were mixed with 50 nM rat liver TrxR. The A_{340} was followed against identical blank without DSNA. (B) DSNA (10-25 μM) in 0.2 ml of 50 mM Tris-HCl, 1 mM EDTA, pH 7.4 containing 100 μM NADPH and 6 mM DTNB were mixed with 50 nM rat liver TrxR. The A_{412} was followed against identical blank without DSNA.

Figure S6. The cytotoxic effect of DSNA in lymphocytes as determined by the MTT assay. The cells were treated with the increasing concentrations of DSNA for 24 h and processed for MTT

assay as described in the method section. Results are presented as mean ± SEM, n = 3. CN – Con troll cells, DC – DMSO control.

Figure S7. Radioprotective effect of DSNA pretreatment (25 μM for 16 h) against the radiation (4 Gy) induced apoptosis in splenic lymphocytes at 24 h post irradiation. (A) DNA ladder assay (B) Quantitative analysis of red/green fluorescence intensity ratio after JC1 staining (C) The mRNA expressions of *Bcl2* and *Bax* genes as determined by RT-PCR analysis. The expressions of above genes in different treatment groups were normalized against the sham control group and the relative expression changes have been plotted. Actin expression was used as internal control. Results are presented as mean- ± SEM, n = 3. *$p < 0.05$ as compared to the control. #$p < 0.05$ as compared to the radiation control. CN – Control, RC – Radiation control

Figure S1

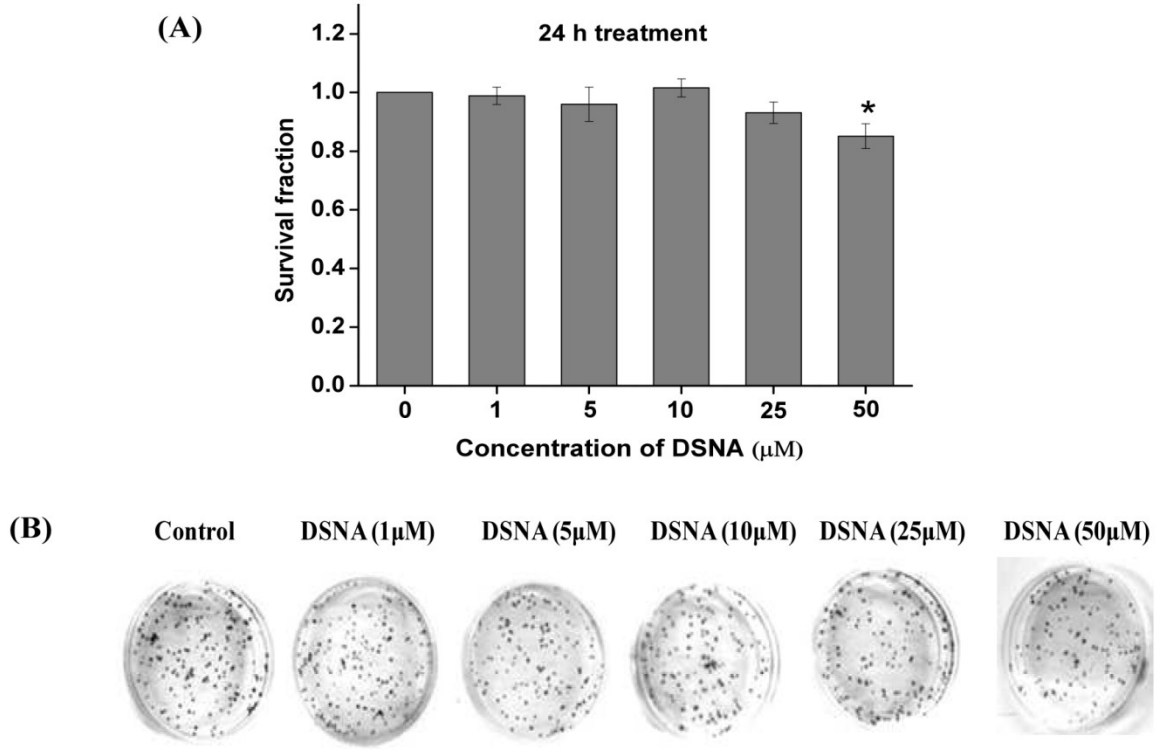

(A)
24 h treatment
Survival fraction
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
0
1
5
10
25
50
Concentration of DSNA (μM)
(B)
Control
DSNA (1μM)
DSNA (5μM)
DSNA (10μM)
DSNA (25μM)
DSNA (50μM)

Figure S2

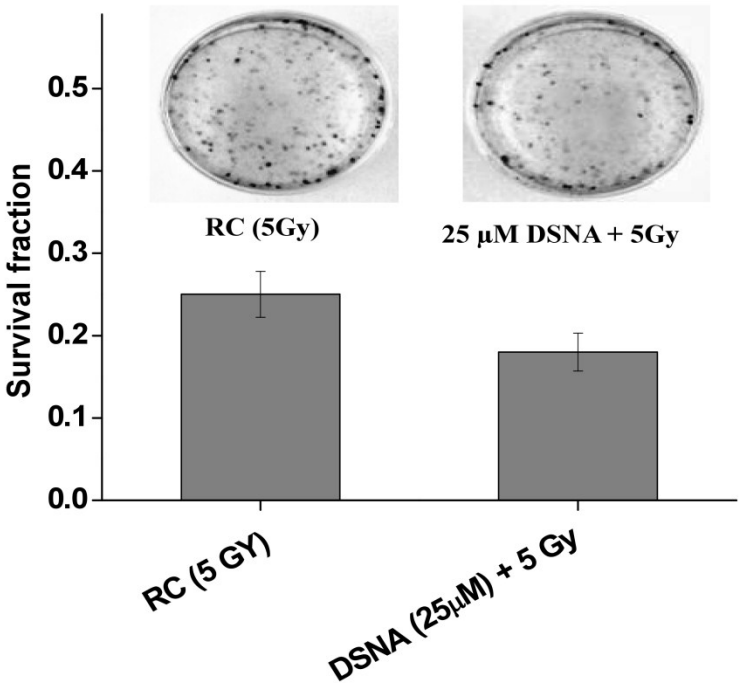

RC (5Gy)
25 μM DSNA + 5Gy
Survival fraction
0.5
0.4
0.3
0.2
0.1
0.0
RC (5 GY)
DSNA (25μM) + 5 Gy

Figure S3

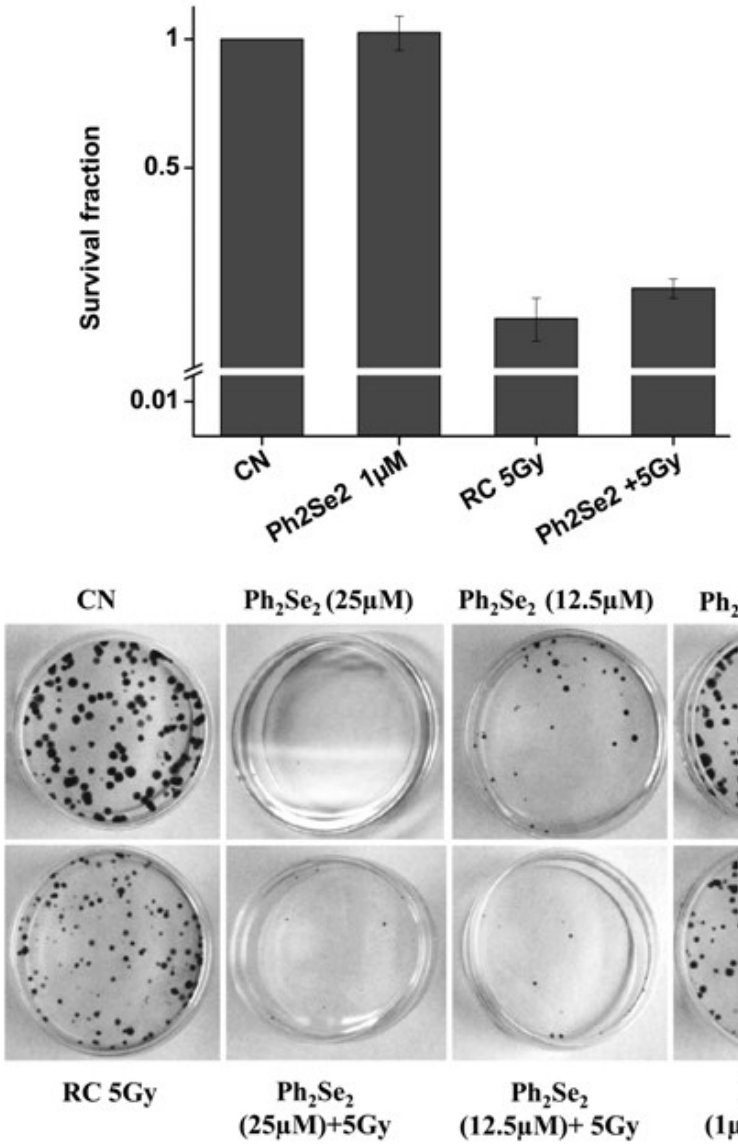

Survival fraction
1
0.5
0.01
CN
Ph2Se2 1μM
RC 5Gy
Ph2Se2 +5Gy
CN
Ph2Se2 (25μM)
Ph2Se2 (12.5μM)
Ph2Se2 (1μM)
RC 5Gy
Ph2Se2 (25μM)+5Gy
Ph2Se2 (12.5μM)+ 5Gy
Ph2Se2 (1μM)+ 5Gy

Figure S4

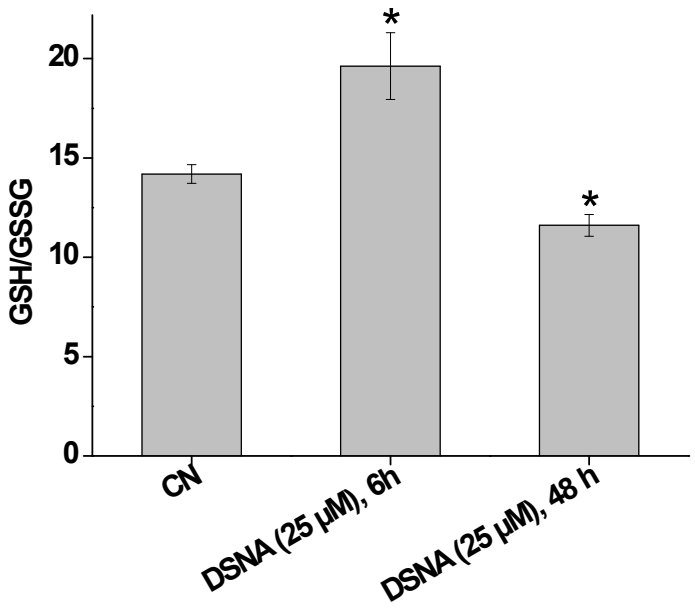


Figure S5.

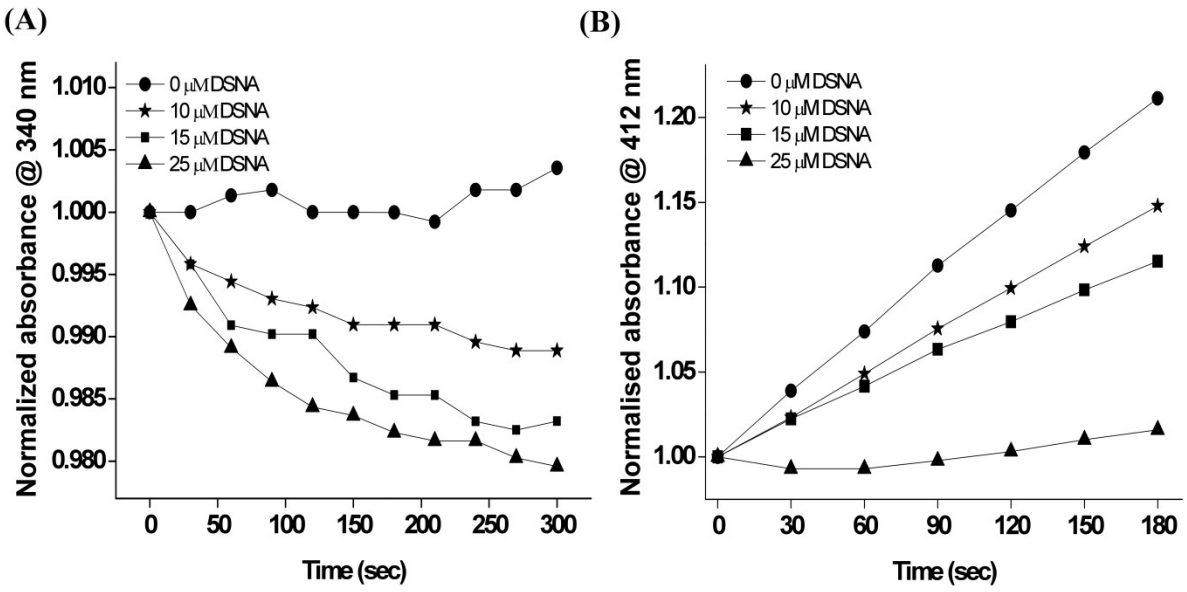

(A)
Normalized absorbance @ 340 nm
1.010
1.005
1.000
0.995
0.990
0.985
0.980
0 μM DSNA
10 μM DSNA
15 μM DSNA
25 μM DSNA
0
50
100
150
200
250
300
Time (sec)
(B)
Normalised absorbance @ 412 nm
1.20
1.15
1.10
1.05
1.00
0 μM DSNA
10 μM DSNA
15 μM DSNA
25 μM DSNA
0
30
60
90
120
150
180
Time (sec)

Figure S6

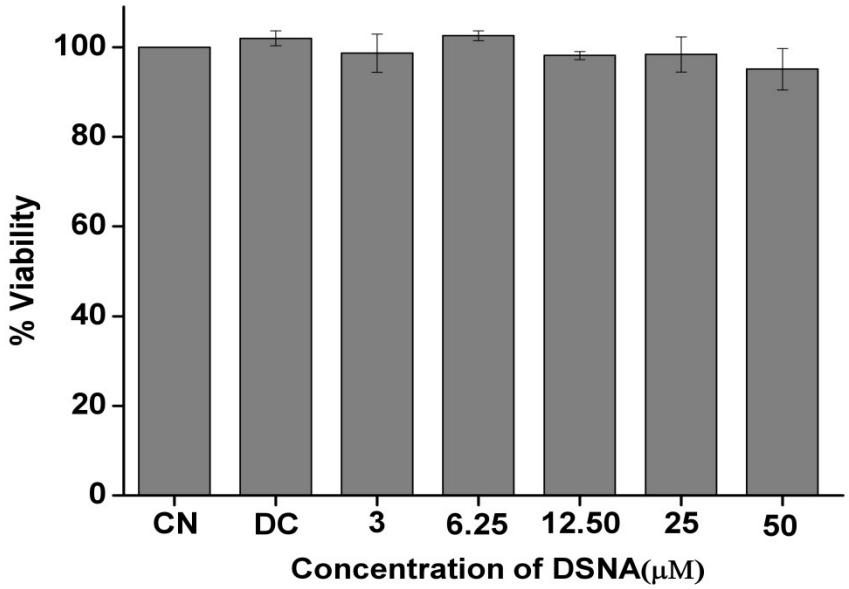
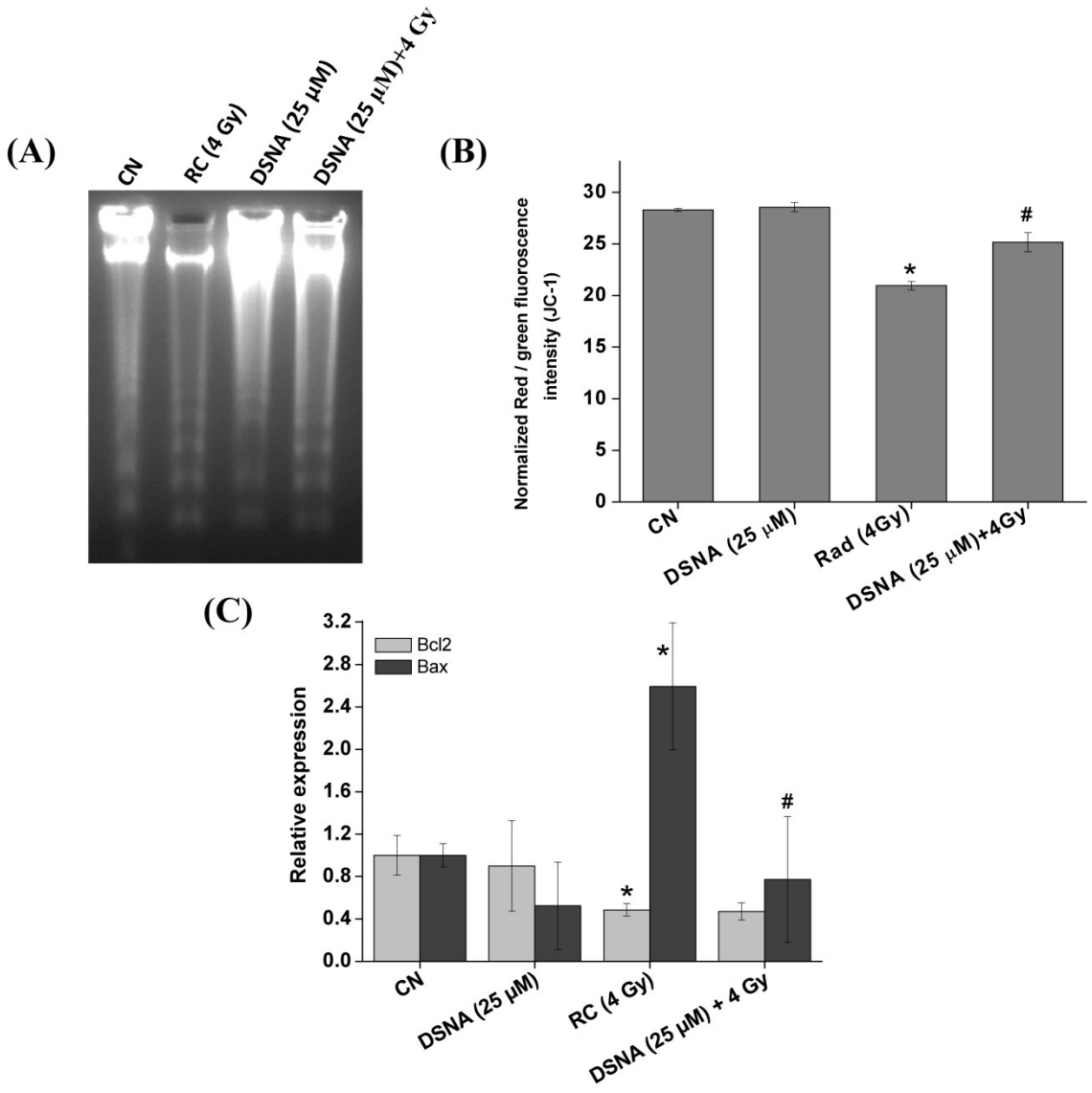

% Viability
100
80
60
40
20
0
CN
DC
3
6.25
12.50
25
50
Concentration of DSNA(μM)

Figure S7


(A)
CN
RC (4 Gy)
DSNA (25 μM)
DSNA (25 μM)+4 Gy
(B)
Normalized Red / green fluorescence intensity (JC-1)
0
5
10
15
20
25
30
*
#
CN
DSNA (25 μM)
Rad (4Gy)
DSNA (25 μM)+4Gy
(C)
Bcl2
Bax
Relative expression
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
*
*
#
CN
DSNA (25 μM)
RC (4 Gy)
DSNA (25 μM) + 4 Gy