

# Modulation of Radioresponse by an Isoquinoline Alkaloid Berberine in Cultured HeLa Cells Exposed to Different Doses of $\gamma$ -Radiation



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## Abstract

The radiomodulatory effect of berberine chloride (BCL) was studied in cultured HeLa cells exposed to  $\gamma$ -rays, where optimum treatment duration of BCL for its radiosensitizing activity was determined by treating HeLa cells with BCL for different times before exposure to 2Gy  $\gamma$ -rays. Exposure of HeLa cells to BCL for 4h before exposure to 2Gy, caused a significant decrease in the cell viability (50%) and surviving fraction (0.42) after 4h treatment with 4 $\mu$ g/ml BCL. The radiosensitizing activity was studied by treating HeLa cells for 4h with different concentrations of BCL before exposure to 0, 0.25, 0.5, 1, 2, 3, or 4Gy  $\gamma$ -rays. Exposure of HeLa cells to different doses of  $\gamma$ -rays resulted in a dose dependent decline in the viability of HeLa cells, while treatment of HeLa cells with various concentrations of BCL further decreased the cell viability and clonogenic potential of cells depending not only on the irradiation dose but also BCL concentration dependent manner. The lowest cell viability and clonogenicity was observed for 4 $\mu$ g/ml BCL for all exposure doses. The analysis of lactate dehydrogenase release in HeLa cells before exposure to 3Gy irradiation at various post-irradiation times, showed a time dependent elevation in its release up to 12h post-irradiation. Our study demonstrates that BCL treatment increased the effect of  $\gamma$ -rays and this may be due to membrane damage as indicated by lactate dehydrogenase release.

**Keywords:** Berberine; HeLa cells; Survival; Radiation; Lactate dehydrogenase

## Introduction

Ionizing radiations are used regularly to treat various tumors since the discovery of X-rays by Wilhelm Conrad Röntgen in the year 1895 and approximately 40% of patients are cured by radiotherapy and 50% of the patients receive radiotherapy during their disease [1,2]. It is used to treat tumors or as an adjuvant or neoadjuvant therapy and in several instances it is the only modality to treat tumors [3]. It is also used as a palliative therapy if the treatment is impossible [4,5]. Despite the success of radiotherapy its full potential remains underutilized due to the presence of hypoxic fraction of cells in the tumor and also due to the fact that with the increase in the radiation dose the normal tissue radiotoxicity increases [6]. The normal tissue toxicity can be alleviated and the tumor response to radiotherapy may be increased by combining certain drugs with radiotherapy.

There has been an enthusiasm initially to combine hypoxic cell sensitizers with radiotherapy due to its success in preclinical models however; the clinical success has been limited. This has led to development of alternative approach, where chemotherapeutic

drugs were combined with radiotherapy. This approach met with a remarkable success in clinical situation to treat different types of malignant tumors [7,8]. The modern chemotherapeutic drugs such as 5-fluorouracil, cis-dichlorodiammine-platinum (II), cryptophycins, mitomycin C, docetaxel, paclitaxel, camptothecin, irinotecan, topotecan, combretastatin A-4, and gemcitabine have been successfully combined with radiotherapy to treat several neoplasia [8-16]. The chemoradiotherapy has been beneficial in the treatment of solid tumors in many randomized clinical trials, however, the main drawback has been increased high toxicity and development of second malignancies [17,18]. This indicates the need for newer paradigms to reduce the toxic side effects of combination treatment regimens, and extract optimum therapeutic benefits to the patients with good quality of life.

Berberine containing plants are used medicinally in virtually all traditional medical systems, and have a history of long usage in Ayurvedic and Chinese systems of medicine, dating back at least 3,000 years. Berberine is an isoquinoline alkaloid present in a number of clinically important medicinal plants, including

*Hydrastis canadensis* (goldenseal), *Coptis chinensis* (coptis or golden thread), *Berberis aquifolium* (Oregon grape), *Berberis aristata* (tree turmeric) and *Tinospora cordifolia* (giloy). Many useful activities of berberine including antibacterial, antifungal, anticancer, antidiarrheal, antioxidant, anti-inflammatory, antiarrhythmic, antihypertensive, chemosensitizing and hepatoprotective have been reported [19-21]. Berberine has been found to have a conducive effect on memory impairment in rats and also block inflammatory colitis in mice [22,23]. Berberine induced apoptosis by activation of Bax and caspase 3 and inhibited the growth of prostate cancer cells in vitro in an earlier study [24]. Berberine afforded protection against Alzheimer's disease, diabetes, diarrhea, hyperlipidemia, fatty liver disease, gastroenteritis, coronary artery diseases, hypertension, obesity, metabolic syndrome, and polycystic ovary [23,25]. Clinically it has been found to improve cardiac performance in patients with heart failure and exert a direct depressive action on myocardial vasculature and smooth musculature [26]. The administration of 1.2 to 2g of berberine daily in the patients suffering from chronic congestive heart failure has been reported to improve left ventricular ejection fraction and ventricular premature complexes [27]. Clinically berberine treatment has been reported to be effective in type 2 diabetes in humans [28-30]. Berberine has been reported to be clinically effective in the treatment of Behcet's disease, dementia, dyslipidemia, non-fatty liver disease, hyperlipidemia, and ocular disorders [31-36]. Berberine has been reported to trigger molecular damage into the DNA of cultured HeLa cells and also augment the effect of radiation [37,38]. Therefore, the present study was conceptualized to obtain an insight into the radiomodulatory activity of berberine chloride in cultured HeLa cells.

### Materials and Methods

#### Preparation of drug

Berberine chloride (BCL) was dissolved in sterile double distilled water (MEM) at a concentration of 5mg/ml and diluted in MEM in such a way so as to obtain the required concentrations. All drug solutions were prepared afresh immediately before use.

#### Cell line and culture

HeLa S3 cells (doubling time of  $20 \pm 2$ h) were supplied by National Centre for Cell Science, Pune, India. The cells were usually grown in 25cm<sup>2</sup> culture flasks (Techno Plastic Products, Trasadingen, Switzerland) in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% L-glutamine and 50µg/ml gentamicin sulfate at 37 °C in an atmosphere of 5% CO<sub>2</sub> in 95% humidified air in a CO<sub>2</sub> incubator (NuAir, Plymouth, USA) with loosened caps.

#### Experimental design

A fixed number ( $5 \times 10^5$ ) of exponentially growing cells were seeded into several culture flasks (Techno Plastic Products, Trasadingen, Switzerland) and divided into the following groups according to the treatment they received:

**MEM+irradiation:** The cells of this group were cultured in minimum essential medium and exposed to different doses of  $\gamma$ -rays.

**BCL+irradiation:** This group of cell cultures was treated with different concentrations of BCL before exposure to various doses of  $\gamma$ -rays.

#### Irradiation

The 25cm<sup>2</sup> flask containing cells were exposed to 0.0, 0.25, 0.5, 1, 2, 3 or 4Gy of Cobalt-60  $\gamma$ -rays from a Telecobalt therapy source (Theratron Atomic Energy Agency, Ontario, Canada). The cell culture flasks were irradiated at a dose of 1Gy/minute at a SSD of 91cm (midpoint of the flask) at room temperature.

#### Determination of optimum time

The optimum time of BCL treatment for radiosensitization of HeLa cells was determined by performing the following experiments, where grouping and other conditions were essentially similar except that the cells were exposed to 2Gy  $\gamma$ -rays after treating the cells with different concentration of BCL.

#### Pratt and Willis assay

The optimum duration for drug exposure before irradiation was evaluated by Pratt and Willis assay [39] in HeLa cells. Briefly,  $1 \times 10^5$  HeLa cells were inoculated into 25cm<sup>2</sup> culture flasks (Cellstar, Greiner, Germany) and grown for 24h. Thereafter, 1, 2 or 4µg/ml BCL was added and cells were incubated for 0, 1, 2, 4 or 6h at 37 °C and were exposed to 2Gy  $\gamma$ -radiation. The drug-containing medium was replaced with a fresh drug-free medium and transferred to CO<sub>2</sub> incubator. After 72h of drug inoculation, the cells were harvested, stained with trypan blue and the cells were counted using a hemocytometer (Labovet microscope, Ernst Leitz, Wetzlar GmbH, Germany). The cells incorporating the dye were considered as dead cells whereas those excluding the trypan blue were considered viable. The results obtained were further confirmed by clonogenic assay.

#### Clonogenic assay

The grouping and other conditions were similar to that described above except that the clonogenicity of cells was estimated by colony forming assay [40], where 200 cells were plated on to several individual culture dishes (Cellstar, Greiner, Germany) containing 5ml drug free medium in triplicate for each drug dose for each group. The cells were exposed to 1, 2 or 4µg/ml BCL for 0, 1, 2, 4 or 6h, respectively before exposure to 2Gy  $\gamma$ -rays. Immediately after irradiation the cells were transferred back to the CO<sub>2</sub> incubator and were allowed to grow for 11 days to form colonies. The resultant colonies were stained with 1% crystal violet in methanol and clusters containing 50 or more cells were scored as a colony. The plating efficiency of cells was determined and the surviving fraction calculated.

#### Determination of radiosensitizing activity

A separate experiment was carried out to determine the radiosensitizing activity of BCL, where the cells were treated

with 0, 1, 2 or 4 µg/ml BCL for 4 h before exposure to 0, 0.25, 0.5, 1, 2, 3 or 4 Gy of γ-rays as described above within five minutes of irradiation, the medium containing the drug was removed from each culture flask of each group and the cells were washed twice with sterile PBS. The cells from each group of flasks were dislodged by trypsin EDTA treatment and divided into two parts.

#### Pratt and Willis assay

One part of the cells i.e.  $1 \times 10^5$  were inoculated into several culture flasks in triplicate and the cytotoxicity of various treatments was determined by Pratt and Willis test [39] as described above.

#### Clonogenic assay

The results obtained from Pratt and Willis assay were confirmed by clonogenic assay as described above, where the cells left after Pratt and Willis assay were inoculated into several petridishes in triplicate for each doses of radiation for each group and left undisturbed for 11 days for colony formation. The colonies thus formed were counted. The plating efficiency of cells was determined and the surviving fraction was fitted on to a linear quadratic model  $SF = \exp(-\alpha D + \beta D^2)$ .

#### Biochemical analysis

In a separate experiment, effect of 0, 1, 2, or 4 µg/ml BCL was studied on the lactate dehydrogenase (LDH) enzyme release in the medium. The grouping and other conditions were similar to that described in the experimental section. The cells were treated with different concentrations of BCL for 4h and exposed to 3Gy. The LDH was estimated at 0, 0.5, 1, 2, 4, 8 and 12h post-irradiation.

#### Estimation of lactate dehydrogenase activity

The estimation of LDH release in the culture medium of above groups was carried out by the earlier described method with minor modifications [41]. The whole medium from each cell culture of each group was removed and collected separately immediately after irradiation (within 5min after irradiation) and was considered 0h after treatment. The cells were fed with a fresh 5ml medium and the above procedure (removal of media) was successively repeated at each assay period (i.e., 0.5, 1, 2, 4, 8 and 12h) until the termination of the experiment. Briefly, the tubes containing media were centrifuged and 50 µl of the medium was transferred into the individual tubes containing Tris-EDTA-NADH buffer followed by 10 min incubation at 37 °C and the addition of pyruvate solution. The absorbance was read at 339nm in a UV-Vis spectrophotometer (UV-260, Shimadzu Corp, Tokyo, Japan) and the data have been expressed as units/litre (U/L).

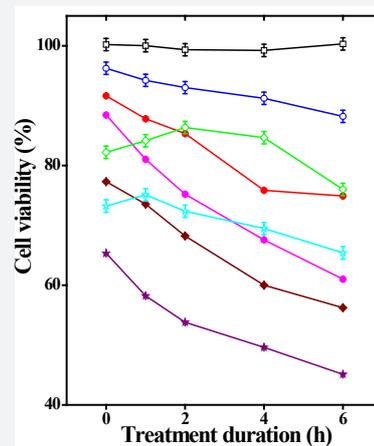
#### Statistical analysis

The statistical analyses were performed using GraphPad Prism 5 statistical software (GraphPad Software, San Diego, CA, USA). The significance among all groups was determined by one-way ANOVA and Bonferroni's post-hoc test was applied

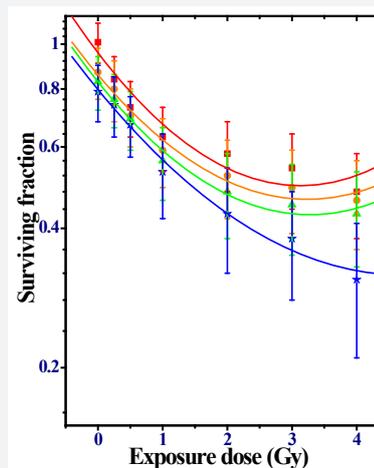
for multiple comparisons. The experiments were repeated for confirmation of results. The results are the average of five individual experiments. The test of homogeneity was applied to find out variation among each experiment. The data of each experiment did not differ significantly from one another and hence, all the values have been combined and means calculated. A p value of <0.05 was considered statistically significant.

### Results

The results are expressed as % viability for Pratt and Willis assay and surviving fraction (SF) for clonogenic assay in (Figure 1 & 2). The results of biochemical analysis are expressed as LDH (units/L) (Table 1) and (Figure 3).



**Figure 1:** Effect of treatment duration of various concentrations of BCL on viability of HeLa cells before exposed to 2Gyγ-irradiation. Open squares: MEM + sham- irradiation; Closed squares: MEM + irradiation; Open circles: 1 µg/ml BCL + sham- irradiation; Closed circles: 1 µg/ml BCL + irradiation; Open diamonds: 2 µg/ml BCL + sham- irradiation; Closed diamonds: 2 µg/ml BCL + irradiation; Open stars: 4 µg/ml BCL + sham-irradiation and Closed stars: 4 µg/ml BCL + irradiation.



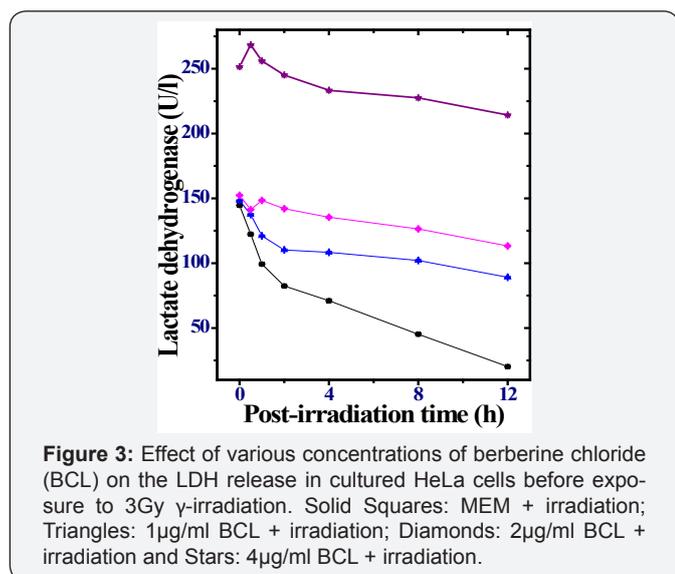
**Figure 2:** Effect of various concentrations of BCL on cell survival in cultured HeLa cells exposed to different doses of γ-rays. Squares: MEM + irradiation; Circles: 1 µg/ml BCL + irradiation; Triangles: 2 µg/ml BCL + Irradiation and Stars: 4 µg/ml BCL + irradiation.

**Table 1:** Alteration in the radiation-induced LDH release in HeLa treated with different concentrations of BCL before exposure to 3Gy  $\gamma$ -rays at different post-irradiation times.

Post-IR Time (H)	Lactate Dehydrogenase (LDH) $\pm$ SEM U/L							
	MEM		Berberine ( $\mu$ g/mL)					
	SIR	IR	1		2		4	
	SIR	IR	SIR	IR	SIR	IR	SIR	IR
0	43.21 $\pm$ 0.33	144.57 $\pm$ 0.35	71.10 $\pm$ 0.41	148.34 $\pm$ 0.34	95.32 $\pm$ 0.34	152.35 $\pm$ 0.43	149.05 $\pm$ 0.32	251.51 $\pm$ 0.50
0.5	43.22 $\pm$ 0.34	122.34 $\pm$ 0.41	68.25 $\pm$ 0.43	137.57 $\pm$ 0.42	88.64 $\pm$ 0.35	141.35 $\pm$ 0.45	134.24 $\pm$ 0.29	268.44 $\pm$ 0.41
1	43.21 $\pm$ 0.33	99.25 $\pm$ 0.35	72.93 $\pm$ 0.41	121.06 $\pm$ 0.41	91.52 $\pm$ 0.31	148.41 $\pm$ 0.52	143.15 $\pm$ 0.31	256.03 $\pm$ 0.43
2	43.195 $\pm$ 0.36	82.41 $\pm$ 0.39	54.15 $\pm$ 0.45	110.23 $\pm$ 0.38	77.05 $\pm$ 0.24	142.04 $\pm$ 0.41 <sup>a</sup>	138.64 $\pm$ 0.29	245.15 $\pm$ 0.50 <sup>a</sup>
4	43.20 $\pm$ 0.34	71.04 $\pm$ 0.44	37.24 $\pm$ 0.36	108.33 $\pm$ 0.42 <sup>a</sup>	48.78 $\pm$ 0.35	135.39 $\pm$ 0.55 <sup>a</sup>	124.43 $\pm$ 0.34	233.36 $\pm$ 0.52 <sup>a</sup>
8	43.21 $\pm$ 0.36	45.22 $\pm$ 0.42	33.45 $\pm$ 0.37	102.11 $\pm$ 0.39 <sup>a</sup>	34.54 $\pm$ 0.31	126.41 $\pm$ 0.40 <sup>a</sup>	86.84 $\pm$ 0.41	227.56 $\pm$ 0.29 <sup>a</sup>
12	44.19 $\pm$ 0.34	20.24 $\pm$ 0.36	20.38 $\pm$ 0.37	89.12 $\pm$ 0.35 <sup>a</sup>	22.35 $\pm$ 0.36	113.33 $\pm$ 0.38 <sup>a</sup>	64.43 $\pm$ 0.42	214.33 $\pm$ 0.34 <sup>a</sup>

a = p<0.001 (Comparison of BCL concentrations with DDW + IR). SIR: Sham Irradiation (0Gy); MEM: Minimum Essential Medium; BCL: Berberine Chloride.

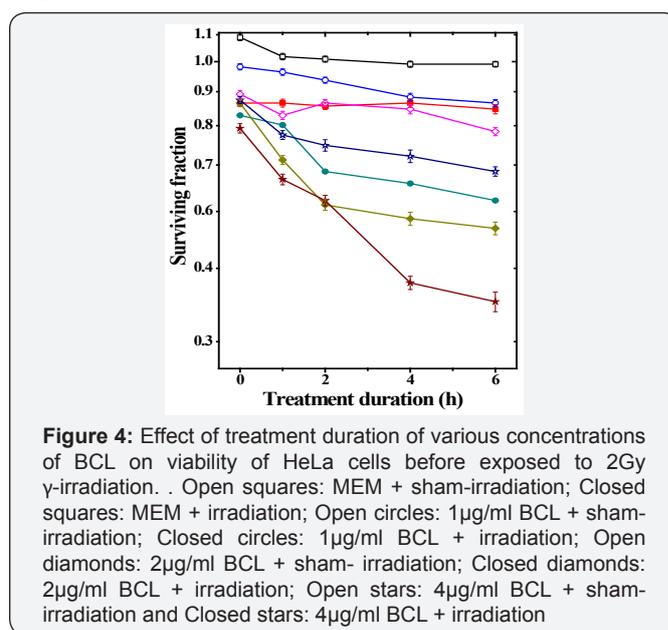
decline thereafter however difference between 4 and 6h was not statistically significant (Figure 1).



### Determination of optimum time

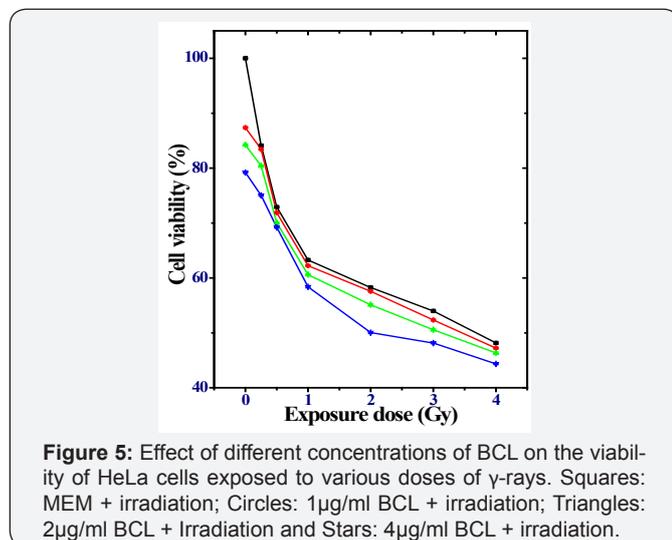
**Pratt and Willis assay:** MEM did not alter the spontaneous viability of HeLa cells with estimation time. However, treatment of HeLa cells with 1, 2 or 4 $\mu$ g/ml reduced the cell viability in a concentration and time dependent manner (Figure 1). Irradiation of HeLa cells with 2Gy  $\gamma$ -rays depleted the cell viability and the cytotoxic effect was almost similar at different MEM treatment durations. Treatment of HeLa cells with 1, 2 or 4 $\mu$ g/ml BCL for different times reduced the cell viability in a concentration and time dependent manner and highest cytotoxic effect was discernible for 4 $\mu$ g/ml BCL for 6h treatment duration (Figure 1). Treatment of HeLa cells with BCL for 4h caused a significant decrease in the cell viability (approximately 50%) and a gradual

**Clonogenic assay:** The reproductive integrity of HeLa cells remained unaffected in MEM+irradiation group with time, as evidenced by non-significant changes in the survival of HeLa cells (Figure 4). Treatment of HeLa cells with 1, 2 or 4 $\mu$ g/ml BCL for various time periods before exposure to 2Gy exhibited a time and BCL concentration dependent decrease in the SF. The SF of HeLa cells treated with 2 $\mu$ g/ml BCL for 4h before irradiation reduced to 0.54 and, thereafter, clonogenicity of HeLa cells declined negligibly with exposure time up to 6h post-treatment, the last BCL treatment time evaluated (Figure 4). Therefore further studies were carried out using 4h treatment time for BCL.



### Determination of radiosensitizing activity

Pratt and Willis assay: MEM treatment did not alter the spontaneous viability of HeLa cells significantly (Figure 5). When HeLa cells were treated with different concentrations of BCL for 4h, the cell viability declined in a concentration dependent manner and a lowest viability was observed for 4 $\mu$ g/ml, the highest concentration of BCL evaluated (Figure 5). Exposure of HeLa cells to different doses of  $\gamma$ -rays resulted in a dose dependent decline in the cell viability and lowest viable cells were recorded for 4Gy  $\gamma$ -irradiation. Treatment of HeLa cells with various concentrations of BCL before exposure to different doses of  $\gamma$ -rays led to a further decrease in the cell viability depending not only on the irradiation dose but also on the concentration of BCL (Figure 5). The lowest dose of 1 $\mu$ g/ml BCL increased cytotoxic effect of  $\gamma$ -rays very significantly when compared with the non-drug treated irradiated control. Exposure of HeLa cells to 2  $\mu$ g/ml BCL further decreased the cell viability at all exposure doses in comparison with MEM + irradiation group where an approximate 2 fold decline in the cell viability was observed for 2 and 3Gy, respectively. The increase in irradiation dose up to 4Gy resulted in a 3 folds rise in cell death in the cultures receiving 2 $\mu$ g/ml BCL. A subsequent increase in BCL concentration up to 4 $\mu$ g/ml, before exposure to different doses of  $\gamma$ -rays further increased the radiation-induced cytotoxicity of HeLa cells, which was maximum among all three BCL doses studied. The effect of BCL treatment was always greater than that of non-drug treated group (Figure 5).



**Clonogenic assay:** Sham irradiation did not alter cell survival after MEM treatment, whereas treatment of HeLa cells with different concentration of BCL resulted in a concentration dependent attrition in cell survival (Figure 2). Exposure of HeLa cells to 0 to 4Gy  $\gamma$ -rays resulted in a radiation-dose dependent depletion in the cell survival and the greatest reduction in cell survival was recorded for 4Gy exposure (Figure 2). A similar pattern was observed in HeLa cells treated with different concentrations of BCL, except that the clonogenicity of cells

declined significantly with BCL concentration when compared with MEM + irradiation group. The lowest SF was observed for 4 $\mu$ g/ml BCL for all irradiation doses. This reduction in the clonogenic potential of HeLa cells was always greater than MEM pretreatment (Figure 2).

### Lactate dehydrogenase

Irradiation of HeLa cells with 3Gy  $\gamma$ -rays caused an elevation in LDH release in the medium when compared to sham-irradiated controls (Table 1). Treatment of HeLa cells with various concentrations of BCL before irradiation elevated LDH significantly when compared to 3Gy  $\gamma$ -ray exposure. The LDH activity was highest immediately after irradiation (0h) in all the groups. However, this elevation was 2 folds greater at other post-irradiation assay times in BCL + IR group (Table 1 and Figure 3). The LDH release declined with assay time (since the whole media was removed at each time, the values in tables and graphs are lower) reaching a nadir at 8h post-irradiation (Figure 3), however, the LDH contents were significantly higher ( $p < 0.001$ ) than the sham-irradiated control (MEM + 0Gy) as well as MEM + 3Gy irradiation group for all BCL concentrations (Table 1).

### Discussion

Radiotherapy and chemotherapy have multiple negative effects on the immune system, as do surgery, anesthetics, antibiotics, blood transfusions and all drug and chemical exposures that may form part of cancer treatment. The inclusion of a drug in radiotherapy regimens will improve the therapeutic index by killing neoplastic cells and reducing radiation toxicity to normal tissues [42]. Our earlier study has shown that 10mg/kg b.wt. berberine chloride (BCL) killed neoplastic cells in vivo [21] and reports regarding the systematic evaluation of radiosensitizing activity of BCL in vitro are lacking. Therefore the present study was undertaken to study the radiosensitizing activity of berberine in cultured HeLa cells exposed to different doses of  $\gamma$ -rays.

Treatment of HeLa cells with different concentrations of BCL before irradiation caused radiation dose-dependent decline in the cell survival. Berberine has been reported to increase the radiosensitivity of prostate cancer cells and xenografted tumor earlier [43]. Our earlier study has shown that berberine treatment enhanced the effect of radiation by triggering molecular DNA damage and cell death [38]. Similarly, the other drugs like vinblastine, chlorpromazine, bleomycin, vincristine, teniposide, taxol, adriamycin, vindesine and echitamine have been reported to increase the effect of radiation in vitro and in vivo [44-48]. Treatment of HeLa cells with various concentrations of BCL caused a significant decline in cell viability after exposure to 1 to 4Gy  $\gamma$ -radiation. The highest concentration of 4 $\mu$ g/ml BCL increased cytotoxic effect of  $\gamma$ -radiation significantly when compared with the non-drug treated control. A similar effect has been observed for V79 cells treated with taxol, vindesine and teniposide before irradiation [49-51]. Acyclovir and

azidothymidine have also been found to enhance the effect of radiation in HeLa cells [52,53].

BCL treatment increased the radiation-induced LDH release significantly at all post-irradiation times. The measurement of LDH release is useful in assessing the cytotoxicity of cells in cases of advanced cell membrane damage [54]. An increase in LDH contents after paclitaxel and VM-26 treatment has been reported earlier [45]. The increased LDH activity is closely related to the reduced surviving fraction in the present study. A direct correlation between increase in LDH release and a consequent decline in cell survival has been reported earlier [45,51,55,56].

The mechanisms involved in the increased radioresponse by BCL in the present investigation may not be ascribed to a single mechanism, but multiple mechanisms may be acting in concert with other to increase the radiosensitivity of HeLa cells. The ionizing radiations interact with cellular aqueous milieu by triggering formation of free radicals [57,58]. The combination of berberine with radiation would have enhanced the free radical formation as berberine has also been reported to produce free radicals [59]. This free radical production would have damaged DNA causing increased cell death. Our earlier studies have shown that berberine induces molecular DNA damage and it also augmented radiation-induced DNA damage [37,38]. The inhibition of DNA topoisomerase-II by berberine may have increased the cell killing effect of radiation as it has been actually found to repress the activity of topoisomerase II in vitro [60]. Acceleration of radiation-induced apoptosis by berberine may have also contributed to increased cell killing effect in BCL + irradiation group. Berberine has been reported to increase the apoptosis in MCF-7 cell by increasing the activity of cytochrome C, caspase 9, PARP cleavage and reducing the activity of Bcl2 [61]. The HeLa cells constitutively express NF- $\kappa$ B and suppression of NF- $\kappa$ B by BCL may have played an important role in increasing the radiosensitivity. BCL has been found to downregulate NF- $\kappa$ B, COX-II, p53, activator protein 1, and cyclins that may have played a very significant role in enhancing the radiosensitivity of HeLa cells in the present study. Inhibition of HIF-1 $\alpha$  and VEGF expression by berberine may have also been responsible for increased radiosensitivity [43]. Apart from the mechanisms described above, increased LDH activity- may have played an important role in increasing the cytotoxicity of radiation.

### Conclusions

Treatment of HeLa cells with BCL before exposure to different doses of  $\gamma$ -rays increased the cytotoxic effect of radiation. The upsurge in the cytotoxic effect of radiation by BCL may be due to inhibition of topoisomerase II activity, NF- $\kappa$ B, COX-II, p53, activator protein 1, HIF-1 $\alpha$  and VEGF and cyclins and increase in LDH release by changing the fluidity of the cell membrane. Apart from these, elevation in radiation-induced DNA damage and apoptosis by BCL may have also contributed to increased cell death after irradiation. The present study in

preclinical model shows that BCL is capable of enhancing the radiosensitivity of HeLa and deserve attention as radiosensitizer in clinical situation, where it may bring out therapeutic gains in the cancer patients.

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