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Research Article

Berberine Chloride, An Isoquinoline Alkaloid Induces Cytotoxicity in Cultured Hela Cells

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Abstract

The chemotherapy is among one of the cancer treatment regimens, which is used frequently either alone or in conjunction with other treatment modalities to treat various neoplastic disorders. However, it causes several side effects and debility in the recipients, which stresses the need to search newer modalities that do not exert the adverse side effects unlike the existing chemotherapy regimens. The cytotoxic effect of Berberine Chloride (BCL), an isoquinoline alkaloid isolated from *Tinospora cordifolia* has been investigated in HeLa cells, where optimum duration of drug treatment was selected by treating Hela cells with 8 µg/ml BCL for 0, 1, 2, 4, 6, 8, 12, 18 or 24 h. The treatment of HeLa cells with BCL for 4 h led to 65% decline in the cell survival and therefore further studies were carried out by exposing the cells with 1, 2, 4, 6 or 8 µg/ml BCL for 4 h. Treatment of HeLa cells with various concentrations of BCL resulted in a concentration dependent reduction in the clonogenicity of HeLa cells as evidenced by a continuous decline in cell survival with increasing concentration of BCL. The lowest surviving fraction of 0.41 was obtained in the cells treated with 8 µg/ml BCL. To understand the mechanism of action we also analyzed the Glutathione-S-Transferase (GST) activity, and Lactate Dehydrogenase (LDH) release at 0, 0.5, 1, 2, 4, 8 and 12 h post-BCL treatment. Exposure of HeLa cells to different concentrations of BCL caused a concentration dependent depletion in GST activity that was lowest at 2 h post-treatment, whereas LDH activity elevated in a concentration dependent manner with a maximum rise at 1 h post-treatment. The BCL treatment killed the cells in a concentration dependent manner and its cytotoxic effect may be due to increase in LDH accompanied by a decline in GST activity.

Keywords: Berberine; Cell Survival; Glutathione-S-Transferase; Hela Cells; Lactate Dehydrogenase

Introduction

The use of synthetic chemicals in the form of drugs started in the nineteenth century by Paul Ehrlich, who treated infectious diseases and also coined the term chemotherapy. However, now the term chemotherapy is synonymous with cancer treatment [1]. The cancer is treated with surgery, ionizing radiations or chemicals agents either alone or in combination with one another. These modalities have advantages and disadvantages. The chemicals that are genotoxic have been used to kill highly proliferating cells as they act on DNA and block cell proliferation and kill the cells more effectively [2]. However, the use of chemical agents to treat cancer is fraught with development of second malignancies in the surviving patients [3]. It is known that despite the development of several modern methods of treatment of cancer, the mortality rates due to solid tumors have not improved appreciably [4], which indicates a need to continue to search newer paradigms to treat cancer.

Medicines derived from plants have played a pivotal role in healthcare of ancient and modern cultures and they have been tested for several generations of humans without untoward adverse side effects. Treatment of different diseases including cancer in Ayurveda, the Indian system of medicine mainly depends on the use of medicinal plants as drugs or drug formulations that mainly contain plants apart from other ingredients. The utility of medicinal plants cannot be underestimated in the healthcare as they have provided several pharmacologically active molecules to treat different diseases including cancer [5,6]. The recent trend shows that less synthetic molecules have come to the market as medicines and

there has been a resurgence of interest in the natural products based drug discovery [5]. The popularity for use of herbal medicines by general public is due to the belief that botanicals will provide some measure of benefit over and above modern allopathic medical approaches [7]. They are also considered non-toxic or less toxic than the synthetic molecules because of their biologic origin and they are time tested in humans.

New compounds possess structures and chemical properties, which suggest that they may act by selective alkylation of growth-regulatory macromolecules. This approach makes a clear case for the value of investigation of plants as means of discovering and developing new anticancer agents [8]. The antitumor activity is encountered in compounds encompassing a wide variety of chemical classes including those synthesized by plants for various purposes. Many of these have novel chemical structures, especially those of considerable complexity, requiring the development of methods for the creation of new ring systems. On the basis of activity against experimental tumors, which is thought to be most predictive of clinical effectiveness, it appears that certain chemical classes including diterpenes, lignans, quassinoids, ansa-macrolides and alkaloids, are of greater interest as a source of anti-neoplastic agents than the others [6,8,9].

Berberine, an isoquinoline alkaloid is synthesized by several plants including *Argemone mexicana, Berberis aristata, Berberis aquifolium, Berberis vulgaris* (barberry) *Coptis chinensis, Coptis japonica, Eschscholzia californica, Hydrastis canadensis, Phellondendron amurense*, *Phellondendron chinense Schneid, Tinospora cordifolia,* and *Xanthorhiza simplicissima* [10,11]. Berberine has been reported to possess antioxidant, anti-inflammatory, antibacterial, antifungal, antiarrhythmic, antihypertensive, antidiarrheal, and hepatoprotective activities [12]. It has been found to inhibit the chemically-induced carcinogenesis in mice and rats and also exert anticancer effect in mice bearing Ehrlich ascites carcinoma at a dose of 10 mg/kg b. wt. [13,14]. Berberine has been reported to protect against memory impairment in rats and also inhibit inflammatory colitis in mice [15,16]. It has been reported to suppress the growth of prostate cancer cells in vitro by inducing apoptosis and activation of Bax and caspase 3 [17]. Berberine has been found to be active against coronary artery diseases, diabetes, diarrhea, fatty liver disease, gastroenteritis, hyperlipidemia, obesity, and hypertension, metabolic syndrome, polycystic ovary and Alzheimer's disease [10,16]. Clinical trials have indicated that berberine is effective in improving the cardiac performance in patients with heart failure and it is presumed to exert a direct depressive action on myocardial vasculature and smooth musculature [18]. Another clinical study on the patients suffering from chronic congestive heart failure, has reported that administration of 1.2 to 2 g of berberine daily in these patients showed improvement in left ventricular ejection fraction and ventricular premature complexes [19]. The berberine has proved its clinical efficacy in the treatment of type 2 diabetes in humans [20-22]. Berberine has also been found to be clinically effective against non-fatty liver disease, dyslipidemia, hyperlipidemia, dementia and ocular Behcet's disease [23-28]. Berberine has been reported to induce molecular damage into the DNA of HeLa cells and also increase the effect of radiation [29,30]. Berberine belongs to the camptothecin family of drugs, which are characterized by their ability to induce DNA topoisomerase II poisoning and hence trigger apoptotic cell death [31]. But, studies regarding the systematic anticancer activity of berberine in HeLa cells are lacking. Therefore, the present study was envisaged to obtain an insight into the anticancer activity of berberine chloride in cultured HeLa cells.

Materials and Methods

Drugs and Chemicals

Berberine Chloride (BCL), fetal calf serum, Minimum Essential Medium (MEM), L-glutamine, gentamicin sulphate, 1-Chloro,2,4-Dinitrobenzene (CDNB) were procured from Sigma Aldrich Chemical Company Ltd., Bangalore, India, whereas other routine chemicals were purchased from Merck India Ltd., Mumbai, India. The doxorubicin hydrochloride was obtained from Biochem Pharmaceutical Industries, Mumbai, India.

Drug Preparation

Berberine Chloride (BCL) and Doxorubicin Hydrochloride (DOX) were freshly dissolved immediately before use in sterile Double Distilled Water (DDW) at a concentration of 5 mg/ml and further diluted in sterile MEM in such a way so as to obtain the required concentrations.

Cell Line and Culture

HeLa S3 cells having a doubling time of 20 ± 2 h were procured from National Centre for Cell Science, Pune, India. The cells were routinely grown in 25 cm^2 culture flasks (Techno Plastic Products, Trasadingën, Switzerland) containing Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum, 1% L-glutamine and 50 µg/ml gentamicin sulfate at 37°C in an atmosphere of 5% CO_2 in 95% humidified air in a CO_2 incubator (NuAir, Plymouth, USA) with their caps loosened.

Experimental Design

Usually $5X10^5$ exponentially growing HeLa cells were seeded into several culture flasks (Techno Plastic Products, Trasadingën, Switzerland) and were divided into the following groups according to the treatment:-

- MEM: The cells of this group were cultured in minimum essential medium.

- BCL: The cell cultures of this group were treated with BCL.

Selection of Optimum Duration

The optimum duration for drug exposure was evaluated in HeLa cells by Pratt and Willis test [32]. Usually $1X10^5$ cells were seeded into 25 cm^2 culture dishes (Cellstar, Greiner, Germany). The cells were allowed to grow for next 24 h and BCL was added at a concentration of 8 µg/ml. The drug-containing medium was replaced with a fresh drug-free medium after 0, 1, 2, 4, 6, 8, 12, 18 or 24 h of BCL treatment. After 48 h of BCL inoculation, the cultures were harvested and the cells were counted under an inverted microscope (Ernst, Leitz GmbH, Wetzlar, Germany). The viability of cells was determined using trypan blue dye-exclusion test.

The results from the Pratt and Willis assay were confirmed by clonogenic assay [33], where 200 cells were plated on to several individual culture dishes (Cellstar, Greiner, Germany) containing 5 ml drug free medium in triplicate for each drug dose for each group. After 24 of plating the cells were exposed to BCL for 0, 1, 2, 4, 6, 8, 12, 18 or 24 h, respectively after which the drug containing medium was replaced with a drug free medium. The cells were left undisturbed in a CO2 incubator for next 11 days for colony formation. The colonies thus formed were stained with 1% crystal violet in methanol and the clusters containing 50 or more cells were scored as a colony. The plating efficiency of cells was determined and the surviving fraction was fitted on to a linear quadratic model = $\exp-\alpha D+\beta D^2$.

Evaluation of Anticancer Activity

The anticancer activity of BCL was evaluated by Pratt and Willis test [32] in HeLa cells, where grouping and other conditons were essentially similar to that described in experimental section above except that the HeLa cells were treated with 1, 2, 4, 6 or 8 µg/ml BCL for 4 h duration and cell viability was evaluated by trypan blue dye exclusion assay. The results from Pratt and Willis assay were confirmed by clonogenic assay [33], where the grouping and other conditions were similar to that described above except that 200 cells were plated in several culture dishes and treated with diffrent concentrations of BCL for four hours. The reproductive integrity of cells was assessed by clonogenic assay as described above.

Biochemical Analyses

A separate experiment was carried out to examine the effect of various concentrations of BCL (0, 1, 2, 4, 6 or 8 µg/ml) on the enzyme activities in the cell homogenate (glutathione-S-transferase) or medium (lactate dehydrogenase) at various post-BCL treatment times. The grouping and other conditions were essentially similar to that described above, except that Doxorubicin (DOX) group was incorporated as a positive control. The enzyme contents released in the medium (LDH) or cell homogenates of all groups were determined at 0, 0.5, 1, 2, 4, 8 and 12 h post-treatment.

Glutathione-S-Transferase (GST)

The cytosolic Glutathione-S-Transferase (GST) activity was determined spectrophotometrically at 37°C according to the procedure of Habig et al. [34]. Briefly, the reaction mixture (2.7 ml of 100 mM phosphate buffer (pH 6.5) and 0.1 ml of 30 mM CDNB was preincubated at 37°C for 5 min, and the reaction was initiated by the addition of 0.1 ml of supernatant and the absorbance was recorded for 5 min at 340 nm in a UV-Visible double beam spectrophotometer (UV-260, Shimadzu Corp, Tokyo, Japan). Reaction mixture without the enzyme was used as a blank. The GST activity has been expressed as U/mg protein.

Lactate Dehydrogenase (LDH)

The activity of LDH was estimated at 0, 0.5, 1, 2, 4, 8 and 12 h post-drug treatment in the culture medium of all the three groups simultaneously by the method described by Decker and Lohman-Matthes [35] with minor modifications. The whole medium from each cell culture of each group was aspirated and collected separately immediately after BCL treatment and was considered 0 h after treatment. The cells were fed with a fresh 5 ml medium and the above procedure (removal of media) was successively repeated at each assay period (i.e., 0.5, 1, 2, 4, 8 and 12 h) 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 addition of pyruvate solution. The absorbance was read at 339 nm on a UV-Visible spectrophotometer (UV-260, Shimadzu Corporation, Tokyo, Japan) and the data have been expressed as units/litre (U/L).

Statistical Analyses

The statistical analyses were performed using GraphPad Prism version 5 statistical software (GraphPad Software, San Diego, CA, USA). The significance among all groups was determined by one-way ANOVA. All the investigations were carried out from the same stock of cells concurrently. 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 data have been combined and means calculated using Microsoft Excel 2000. 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 to 2. The results of biochemical analyses are expressed as; GST, Units / mg protein formed and LDH, units/L in Figure 3-4.

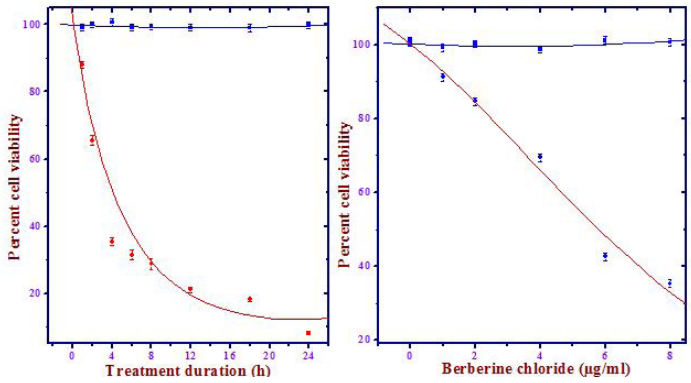

Figure 1: The cytotoxic effect of berberine chloride on HeLa cells. Left: effect of treatment time and right: effect of different concentrations. Closed squares: MEM group; Triangles: berberine chloride group.

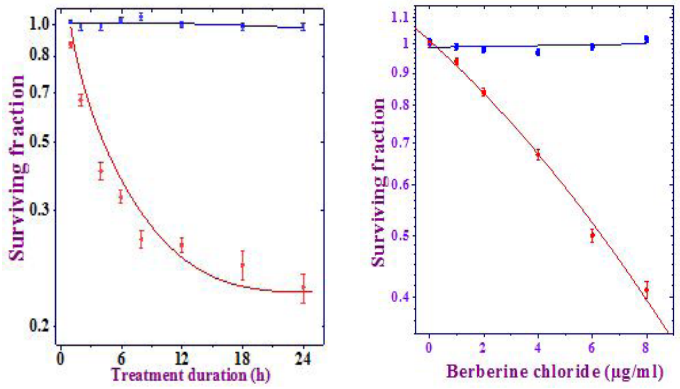

Figure 2: Effect of various concentrations of berberine chloride on the reproductive integrity of cultured HeLa cells. Left Upper:Effect of treatment time and Right Lower: effect of different concentrations of berberine chloride. Closed squares: MEM group; Circles: berberine chloride group.

Selection of Optimum Treatment Duration

Cytotoxicity

MEM treatment did not alter the spontaneous viability of HeLa cells significantly with time (Figure1a). However, treatment of HeLa cells with 8 µg/ml BCL caused a time dependent decline in the cell viability and the lowest viability was observed in the cells exposed to BCL for 24 h (Figure 1a). Exposure of HeLa cells to BCL for 4 h caused a significant decrease in the viability (approximately 65%) of HeLa cells. Thereafter, a gradual decline was observed until cell viability reached a nadir in those cultures that were exposed to BCL for 24 h (Figure 1a).

Clonogenic Assay

The reproductive integrity of HeLa cells remained unaffected by MEM treatment with time, as evidenced by non-significant changes in the survival of HeLa cells (Figure 2a). However, exposure of BCL for various time periods exhibited a time dependent reduction in the clonogenicity of cells as evidenced by a continuous decline in the capacity of HeLa cells to form colonies (Figure 2a). The Surviving Fraction (SF) of HeLa cells reduced up to 0.42 when the cells were in contact with BCL for 4 h. Increase in BCL exposure time caused a steady decline in the clonogenicity of HeLa cells up to 24 h, where the surviving fraction was lowest (Figure 2a).

Evaluation of Anticancer Activity

Cytotoxicity

Exposure of HeLa cells to MEM for 4 h did not alter the spontaneous viability significantly (Figure 1b). However, the viability of HeLa cells exposed to different concentrations of BCL for 4 hours exhibited a gradual but concentration dependent decline with increasing concentration. The lowest viability (approximately 35 %) of HeLa cells was recorded for 8 µg/ml BCL, the highest concentration of BCL evaluated (Figure 1b).

Clonogenic Assay

The reproductive integrity of HeLa cells remained unaffected by MEM treatment as evidenced by non-significant changes in the survival of HeLa cells (Figure 2b). Treatment of HeLa cells with various concentrations of BCL for 4 hours resulted in a concentration dependent decline in the clonogenicity of HeLa cells as evidenced by a steady decline in the cell survival with increasing concentration of BCL (Figure 2b). The lowest surviving fraction of 0.41 was obtained for 8 µg/ml BCL (Figure 2b). The IC50 was found to be approximately 6.4 µg/ml. The cytotoxic effect of BCL was approximately 1.4, 1.8 and 2 folds greater for 4, 6 and 8 µg/ml BCL, respectively.

Biochemical Analyses

MEM treatment did not alter the spontaneous levels of GST and LDH activities significantly, with assay time.

Glutathione-S-Transferase (GST)

The spontaneous activity of GST remained almost unaltered with assay time as no signficant changes were recorded in its activity with assay time (Figure 3). Treatment of HeLa cell with different concentrations of BCL led to a consistant but non-significant decline (Table 1) in the cytosolic GST actvity until a maximum reduction was reached for 8 µg/ml (Figure 3). Similarly, GST actvity decreased with assay time in the HeLa cells treated with diffrent concentrations of BCL and a greatest decline was observed at 12 h post-treatment for all the concentrations of BCL (Figure 3). The pattern of decline in GST activity after DOX treatment was similar to that of BCL, where the decline was also non-significant (Table 1).

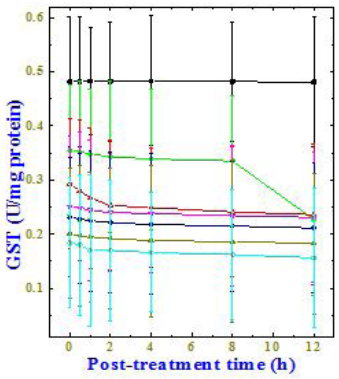

Figure 3: Effect of various concentrations of Berberine Chloride (BCL) glutathione-S-transferase in cultured HeLa cells. Closed squares: Double Distilled Water (DDW); Closed circles: Doxurubicin Hydrochloride (DOX); Open triangles: 1 µg/ml BCL; Open inverted triangles: 2 µg/ml BCL; Closed diamonds: 4 µg/ml BCL; Closed triangles: 6 µg/ml BCL; Open circles: 8 µg/ml BCL.

Post-treatment time	Glutathione-S-Transferase (GST) ± SEM U/mg protein						
(h)	MEM	DOX	BCL (µg/ml)				
	10 µl	10 µg/ml	1	2	4	6	8
0	0.482 ± 0.12	0.355 ± 0.12	0.293 ± 0.12	0.251 ± 0.13	0.231 ± 0.11	0.201 ± 0.12	0.184 ± 0.12
0.5	0.482 ± 0.12	0.352 ± 0.13	0.280 ± 0.13	0.248 ± 0.14	0.228 ± 0.12	0.197 ± 0.13	0.180 ± 0.13
1	0.483 ± 0.10	0.348 ± 0.12	0.267 ± 0.13	0.244 ± 0.13	0.225 ± 0.13	0.195 ± 0.12	0.175 ± 0.14
2	0.482 ± 0.11	0.343 ± 0.14	0.253 ± 0.12	0.240 ± 0.11	0.221 ± 0.13	0.192 ± 0.13	0.171 ± 0.13
4	0.483 ± 0.12	0.339 ± 0.13	0.249 ± 0.11	0.238 ± 0.11	0.218 ± 0.13	0.188 ± 0.14	0.166 ± 0.11
8	0.482 ± 0.11	0.331 ± 0.12	0.241 ± 0.12	0.234 ± 0.13	0.215 ± 0.12	0.186 ± 0.15	0.162 ± 0.12
12	0.481 ± 0.12	0.227 ± 0.14	0.236 ± 0.13	0.231 ± 0.12	0.211 ± 0.12	0.183 ± 0.13	0.156 ± 0.13
Note: The decline in the GST activity was statistically non-significant when compared to MEM group or comparisons were made among various concentration and different post-treatment times.							

Table 1: Alteration in the Glutathione-S-Transferase (GST) activity in HeLa cells treated with various concentrations of berberine chloride at different post-treatment times.

Lactate Dehydrogenase (LDH)

The spontaneous activity of LDH did not change with assay time indicating the healthy status of cells (Figure 4). However, the exposure of HeLa cells to various concentrations of BCL elevated the LDH release gradually with time and a maximum release was observed at 1 h post-treatment (since the whole media was removed at each time, the values in tables and graphs are lower with subsequent assay periods). The exposure of HeLa cells to BCL caused a concentration dependent but significant rise in the LDH activity (Table 2) and the maximum elevation in LDH was recorded for 8 µg/ml BCL at all post treatment assay times (Figure 4). Exposure of HeLa cells to DOX elevated the LDH contents gradually with time and a maximum activity was observed at 12 h post-treatment. The pattern of LDH release after BCL treatment was similar to that of DOX treatment except that it was 1.2 folds higher when compared with the DOX group (Figure 4).

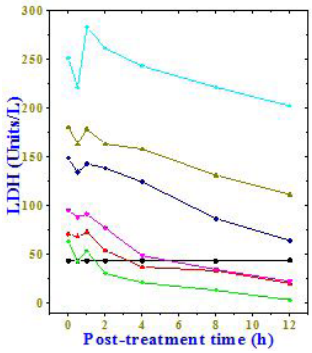

Figure 4: Effect of various concentrations of Berberine Chloride (BCL) on lactate dehydrogenase release in cultured HeLa cells. Closed squares: Double Distilled Water (DDW); Closed circles: Doxurubicin Hydrochloride (DOX); Open triangles: 1 µg/ml BCL; Open inverted triangles: 2 µg/ml BCL; Closed diamonds: 4 µg/ml BCL; Closed triangles: 6 µg/ml BCL; Open circles: 8 µg/ml BCL.

Post-treatment time	Lactate dehydrogenase (LDH) ± SEM U/L						
(h)	MEM	DOX	BCL (µg/ml)				
	10 µl	10 µg/ml	1	2	4	6	8
0	43.21 ± 0.33	63.25 ± 0.35	71.10 ± 0.41	95.32 ± 0.34	149.05 ± 0.32	180.64 ± 0.31	251.21 ± 0.41
0.5	43.22 ± 0.34	73.28 ± 0.36	68.25 ± 0.43	88.64 ± 0.35	134.24 ± 0.29	163.34 ± 0.31	221.42 ± 0.29
1	43.21 ± 0.33	53.64 ± 0.41	72.93 ± 0.41	91.52 ± 0.31	143.15 ± 0.31	178.62 ± 0.36	283.34 ± 0.36
2	43.19 ± 0.36	30.54 ± 0.41	54.15 ± 0.45	77.05 ± 0.24	138.64 ± 0.29	163.41 ± 0.31	261.62 ± 0.34
4	43.20 ± 0.34	21.03 ± 0.36	37.24 ± 0.36	48.78 ± 0.35	124.43 ± 0.34	158.34 ± 0.34	243.29 ± 0.36
8	43.21 ± 0.36	13.26 ± 0.32	33.45 ± 0.37	34.54 ± 0.31	86.84 ± 0.41	131.22 ± 0.35	221.64 ± 0.37
12	44.19 ± 0.34	3.33 ± 0.34	20.38 ± 0.37	22.35 ± 0.36	64.43 ± 0.42	111.45 ± 0.40	202.48 ± 0.38
Note: the data are statistically significant at $p<0.0001$, when DOX and BCL groups were compared to MEM group and also when comparisons were made among different concentrations of BCL at different assay times.							

Table 2: Alteration in the LDH release by HeLa cells treated with different concentrations of berberine chloride at different post-treatment times.

Discussion

Natural medicines have gained popularity over synthetic drugs in recent years with the belief that they are much safer than the synthetic molecules. This has led to the tremendous growth of phytopharmaceutical usage. While it is known that plant extracts are active against cancer, the standard approach has been to isolate, synthesize and administer the single chemical compound thought to be responsible for anticancer effect [6]. A number of modern anticancer drugs including vinca alkaloids, phodophyllotoxins, taxol, camptothecins that are in clinical use have been isolated from different plants [6,8]. The plants/natural products will continue to play a crucial role in the discovery of new anticancer pharmacophores. Our earlier reports have shown that *Tinospora cordifolia* or giloe exert anticancer activity in vitro and in vivo. Berberine isoquinoline alkaloid is also present in giloe. Therefore, the present study was undertaken to investigate the cytotoxic effect of berberine in cultured HeLa cells.

Use of Pratt and Willis assay provides a gross indication of cytotoxicity of any pharmacophore, whereas clonogenic assay is a gold standard that can determine the cytotoxic effect of any chemical or physical agent precisely [36]. The results from the present study demonstrate that BCL exerted cytotoxic effect in a concentration dependent manner and 4 h BCL exposure was considered optimum as it reduced the surviving fraction by almost 65 %. A simliar effect was observed in our earlier study [29]. Berberine has been reported to induce cytotxicity and reduce clonogenicity

in FaDu human pharyngeal squamous carcinoma cells [37]. Berberine treatment has been also reported to increase the cytotoxic effect in HepG2 liver cells and Saos-2 and MG-63 osteosarcoma cells in a concentration dependent manner [38,39]. The berberine loaded Janus magnetic mesoporous silica nanoparticles have been found to exert cytotoxic effect on hepatocellular carcinoma cells [40]. It has also been reported to inhibit the proliferation of human uterine leiomyoma cells [41]. Similarly, *Tinospora cordifolia* that has shown the presence of berberine has been found to exert cytotoxic effect on HeLa cells in concentration dependent manner earlier [30,42,43].

The LDH is a cytoplasmic soluble enzyme and it is released from the cells having damaged membranes and provides an indirect measure of cell cytotoxicity that directly correlates with the amount of LDH released in the cell culture medium [44]. In the present study, LDH, an indicator of cell damage was found to increase in a time dependent manner up to 12 h. Berberine has been found to release LDH in a time edependent manner as observed in the present study [45]. An increase in LDH activity has been reported in cultured neonatal cardiomyocytes that has been correlated to cell death [46]. A direct correlation between increase in LDH and a consequent decline in cell survival has been reported earlier [45-49]. The GSTs conjugate GSH to a variety of endo- and exogenic electrophilic substances, and regulate cell demise and proliferation [50]. The decline in GST may have also caused cytotoxic effect. Although the decline was not statistically significant the cytosolic glutathione-S-transferase was found to deplete in HeLa cells, up to 12 h post-treatment BCL, which may have contributed to cell death in the present study.

The exact mechanism of cytotoxicity induced by berberine on HeLa cells is not well understood. However, putative mechanisms of cell death by berberine may be several. The bereberine treatment may cause cytotoxicity by damaging the cellular DNA. The berberine has been found to damage DNA of HeLa cells at molecular level earlier [29,30]. Topoisomerase II is an enzyme that is essential during replication as it acts by passing an intact segment of duplex DNA through a transient double-strand break, which is produced by it in a separate double helix [51] and inhibition of topoisomerase II by BCL may have stabilized the transient DNA double strand breaks leading to cytotoxicity. Berberine has been reported to inhibit topoisomerase-II [10]. The berberine has been reported to kill cells by inducing apoptosis in HeLa cells in a dose dependent manner by upregulating p53 expression and inhibiting the expression of Bcl-2 and COX-II [52] and similar mechanism of action may have contributed to cell death in the present study. The apoptosis induction may be due to its ability to activate caspase 3, 7 and 8. Berberine has been reported to activate caspase 3, 7 and 8, FasL, TNF-related apoptosis-inducing ligands, the proapoptotic factors including Bax, Bad, Apaf-1, and the active form of caspase-9. It has also been reported to suppress the anti-apoptotic factors, such as Bcl-2 and Bcl-xL [37]. The nuclear factor kappa-B (NF-κB) is expressed by cancer cells as it offers survival advantage and HeLa cells also constitutively express NF-κB [53] and the inhibition of NF-κB activation by berberine may have induced cytotoxic effect by activating apoptosis inducing genes as described above. The berberine has been reported to suppress the transactivation of NF-κB earlier [54].

Conclusions

The BCL treatment increased the cytotoxicity of HeLa cells in a concentration dependent manner, which may be due to induction of molecular DNA damage, and inhibition of topoisomerase II enzyme. Its ability to deplete the GST and increase the lactate dehydrogenase release in the HeLa cells indicate that BCL may have multiple negative effects on the cells, like alteration in the membrane fluidity and damage to the cell DNA and thus enabling cell kill. The other important mechanism seems to be induction of apoptosis, which may be due to the ability of BCL to block the transactivation of NF-κB, Bcl-2 and Bcl-xL and activate proapoptotic factors including p53, Bax, Bad, Apaf-1, caspase 3, 7 and 8 and the active form of caspase-9. The report regarding the clinical use of berberine are very few however our study indicates to its clinical utility in cancer treatment.

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