CHAPTER IV

EVALUATION OF APOPTOTIC POTENTIAL OF CURCUMIN AND ITS SOLID LIPID NANOPARTICLES
1.0 INTRODUCTION

Role of curcumin as an anticancer agent is well established in literature (Anand et al., 2008; Bachmeier et al., 2007). It has been shown to protect against different cancers, including leukaemia and lymphoma, gastrointestinal cancers, genitourinary cancers, breast cancer, ovarian cancer, head and neck squamous cell carcinoma, lung cancer, melanoma, neurological cancers and sarcoma, in in vitro studies including cancerous cell lines like HL-60, K562, MCF-7 and HeLa (Aggarwal et al., 2003; Woo et al., 2005; Yoysungnoen et al., 2006; Zheng et al., 2002). This multitargeting ability of curcumin may be the key to its therapeutic anticancer potential which helps modulating as many as 33 different proteins including thioredoxin reductase, cyclooxygenase-2 (COX-2), protein kinase C (PKC), 5-lipoxygenase and tubulin (Aggarwal et al., 2003). Other molecular targets modulated by this agent include transcription factors, growth factors and their receptors, cytokines, enzymes and genes regulating cell proliferation and apoptosis (Aggarwal et al., 2003; Aggarwal et al., 2007b; Aggarwal et al., 2009).

Previous chapter established a significantly enhanced bioavailability (32-155 times) of curcumin achieved with solid lipid nanoparticles using a highly validated LC/MS/MS method (Kakkar et al., 2011b; Kakkar et al., 2010). In a view to observe the effect of prepared lipidic nanoparticles on improvising and maintaining the anticancer potential of curcumin, we conducted apoptotic and mechanistic studies.

Cell cytotoxicity was evaluated after treatment with curcumin and its developed solid lipid nanoparticles (C-SLNs) (2.7-54 μM) in A549, PC3 and human leukemia HL-60 cell lines. Further, a deeper insight into the molecular events leading to C-SLNs induced apoptosis in HL 60 cells were also demonstrated. The observed apoptotic activity of this bioavailable form of curcumin is also evaluated in terms of loss of mitochondrial membrane potential, release of pro-apoptotic factors and activation of caspases, which together account for apoptotic cell death.

2.0 MATERIALS AND METHODS

2.1 Chemicals and antibodies

RPMI-1640 medium, diaminofluoresceine-2-diacetate (DCF-DA), 2',7' dichlorofluorescein diacetate (DCF-DA), rhodamine-123 (Rh-123), s-methylisothiourea (sMIT),
propidium iodide (PI), DNase-free RNase, proteinase K, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Hoechst 33258, diaminofluorescein 2-diacetate (DAF-2DA), eukaryotic protease inhibitor cocktail, N-acetyl cysteine (NAC), iron, penicillin, streptomycin, histopaque-1077 and-1119 were purchased from Sigma chemical Co. Fetal bovine serum was obtained from Gibco Invitrogen Corporation (#16000-044, lot No. 1237517) USA. Annexin V-FITC apoptosis detection kit, Caspase-8, -9 inhibitors and ApoAlert caspases assay kits were from B.D. Clontech, USA. Mouse anti-human antibodies B-Actin (#SC-47778), TNF-R1 (#SC8436), were from Santa Cruz Biotechnology, USA.

Electrophoresis reagents, protein estimation kit and protein markers were purchased from Bio-Rad Laboratories, USA. Hyper film and ECL Plus western blotting detection kit was obtained from Amersham Biosciences, UK.

2.2 Cell culture, growth conditions and treatment

Human promyelocytic leukemia cell line HL-60, were procured from National Centre for Cell Sciences (NCCS), Pune, India. Human lung carcinoma cell line (A549) and prostrate cancer cell lines (PC3) were procured from National Cancer Institute, Frederick, U.S.A. Cells were grown in RPMI-1640/MEM medium containing 10% FCS, 100 unit penicillin/100g streptomycin per ml of medium in CO₂ incubator (Thermo-con Electron Corporation, USA) at 37°C with 98% humidity and 5% CO₂ gas environment. They were further treated with C-SLNs or curcumin dissolved in dimethyl sulfoxide (DMSO) while the untreated control cultures received only the vehicle (DMSO, ≤0.2%).

2.3 Cell proliferation assay using MTT

This assay is a quantitative colorimetric method for determination of cell survival and proliferation. The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan which can be, after solubilisation with DMSO, directly quantified. The absorbance of the formazan directly correlates with the number of viable cells (Bhushan et al., 2007). The cells were plated in 96-well plates at a density of 2.0 x 10⁴ in 200 μl of medium per well. Cultures were incubated with different concentrations of test material and incubated for 24 or 48 h. The medium was replaced with fresh medium containing 100 μg/ml of 3-(4, 5-dimethylthiazol-2-
yl)-2, 5-diphenyltetrazolium bromide (MTT) for 4 h. The supernatant was aspirated and MTT-formazan crystals were dissolved in 100 µl DMSO and the OD of the resulting solution was measured at $\lambda_{540\text{nm}}$ (reference wavelength, $\lambda_{620\text{nm}}$) on ELISA reader (Thermo Labs, USA). Cell growth was calculated by comparing the absorbance of treated versus untreated cells.

2.4 DNA agarose gel electrophoresis

Apoptosis was also assessed by electrophoresis of extracted genomic DNA from cells. Briefly, 2x10^6 cells after various treatments were centrifuged at 100xg for 10 min, and washed in PBS containing 20 mM EDTA. The pellet was lysed in 250 µl of lysis buffer (100 mM NaCl, 5mM EDTA, 10 mM Tris-HCl, pH 8.0, 5% Triton X-100) containing 400 µg/ml DNase-free RNase and incubated at 37°C for 90 min followed by 1 h incubation with proteinase-K (200 µg/ml) at 50°C for 1 h. The DNA was extracted with 150 µl of phenol for 1 min and centrifuged at 13000xg for 2 min. The aqueous phase was further extracted with phenol: chloroform: isoamylalcohol (25:24:1) and centrifuged. DNA was precipitated from aqueous phase by incubating overnight with 3 volumes of chilled alcohol and 0.3 M sodium acetate at 20°C. The precipitate was centrifuged at 13000xg for 10 min. The DNA pellet was washed in 80% alcohol, dried, dissolved in 50 µl TE buffer, mixed in loading buffer and electrophoresed in 1.8% agarose gel at 50 V for 1.5 h in mixture of tris base, acetic acid and EDTA (TAE) buffer.

2.5 DNA content and cell cycle phase distribution

DNA fragmentation constitutes one biochemical hallmark of apoptosis. Thus, measurement of DNA content makes it possible to identify apoptotic cells, to recognize the cell cycle phase specificity and to quantitate apoptosis. For flow cytometry analysis of the relative nuclear DNA content the fluorescent dye propidium iodide (PI), which becomes highly fluorescent after binding to DNA, is most commonly used. After permeabilisation, PI binds to DNA in cells at all stages of the cell cycle, and the intensity with which a cell nucleus emits fluorescent light is directly proportional to its DNA content. The results of the measurement are illustrated in a histogram, where the number of cells (counts) is plotted against the relative fluorescence intensity of PI (FL-2; $\lambda_{\text{em}}$: 585 nm). The histogram reflects the cell
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cycle distribution of the cell population. Staining normal untreated cells with PI, most of the cells are in G_0/G1 phase (DNA content: 2n) and emit light at a uniform frequency, depicted in the prominent G_0/G1 peak of the histogram. Cells treated with the test material were collected, washed in PBS, fixed in 70% cold ethanol and placed at -20°C overnight. Cells were washed with PBS, subjected to proteinase-K and RNase digestion followed by staining of clean nuclear materials (nuclei) with PI using procedures and reagents as described in the instruction manual of the Cycle Test plus™ -DNA reagent kit (Becton Dickinson, USA). The preparations were analyzed for DNA content using BD-LSR flow cytometer. Data were collected in list mode on 10,000 events for FL2-A versus FL2-W. Apoptotic nuclei appear as a broad hypodiploid DNA peak at lower fluorescence intensity compared to nuclei in G_0/G1 phase (Bhushan et al., 2007).

2.6 Measurement of mitochondrial membrane potential

Mitochondrial dysfunction within the apoptotic process is often associated with loss of the mitochondrial inner transmembrane potential. One possibility to visualize alterations in the mitochondrial membrane potential is staining with potentiometric fluorescent dyes. Changes in mitochondrial transmembrane potential (Ψ_m) as a result of mitochondrial perturbation were measured after staining with Rhodamine-123 (Bhushan et al., 2007). Cells after various treatments in 12-well plate were incubated with medium containing Rhodamine-123 (5 μg /ml; stock, 1mg/ml PBS) for 1 h. Cells were washed in PBS and centrifuged at 100xg for 5 min and suspended in sheath fluid. Immediately before analysis, PI (5 μg /ml; stock 1mg/ml PBS) was added to the samples. The intensity of fluorescence from 10,000 events was analyzed in FL-1 channel on flow cytometer.

2.7 Caspase activity assays

Most of the proteolytic cleavages during apoptosis results from the activation of caspases, a family of cysteine-dependent proteases. These enzymes recognize specific tetra- or pentapeptide motifs in their substrates and cleave exclusively on the carboxyl side of aspartate residues. Caspase activation can be measured by applying a synthetic peptide substrate which is coupled to a fluorophore. Cleavage of the fluorogenic substrate by the activated enzyme leads to increased fluorescence.
The generated fluorophore is proportional to the concentration of activated caspase. The liberation of 7-amino-4-trifluoromethyl coumarin (AFC) shows a blue to green shift in fluorescence at an excitation wavelength of 400 nm and an emission wavelength of 505 nm while the liberation of 7-amino-4-methyl-coumarin (AMC) shows blue to green shift at an excitation wavelength of 380 nm and an emission wavelength of 440 nm respectively. Cells (2\times10^6) were incubated with test material for the indicated concentrations and time periods (Bhushan et al., 2007). At the end of treatment cells were washed in PBS and pellet lysed in cell lysis buffer. Activities of caspase-3, -8 and -9 in the cell lysates were determined fluorometrically using BD Apoalert caspase fluorescent assay kits. Caspase-3 and -8 employed fluorochrome conjugated peptides DEVD-AFC and IETD-AFC as substrates, respectively while caspase-9 employed LEHD-AMC. Release of AFC and AMC were assayed according to the instructions provided in the manual by the supplier. Specific inhibitors were used as negative control to determine whether fluorescence intensity changes were specific for the activity of caspases. The peptide based inhibitors used were DEVD-CHO for caspase-3, IETD-fmk for caspase-8 and LEHD-CHO for caspase-9.

2.8 Immunoblot analysis

The protein lysates along with standard protein marker were subjected to discontinuous SDS-PAGE analysis. Proteins aliquots (50 μg) were resolved on SDS-PAGE, run at 60V (PowerPac™ HC High current power supply, BioRad), for 3 h. The resolved proteins were electro transferred to polyvinylidene difluoride (PVDF) membranes (Bio-RAD) in to Western blotting transfer frames in the following manner: sponge-blotting paper-gel-PVDF membrane-western blotting paper-sponge and transfer overnight at 4°C at 30V in transfer buffer. Non-specific bindings of the membrane are blocked by incubation with 5 % non-fat milk in Tris-buffered saline (10 mM Tris-HCl, 150 mM NaCl) containing 0.1% Tween-20 (TBST) for 1 h at room temperature. The blots are probed with respective primary anti-human antibodies for 2 h (1:1000 dilutions) and washed three times with TBST. The blots are then incubated with horseradish peroxidase conjugated respective secondary antibodies for 1 h (1:1000 dilution), washed again three times with TBST. PVDF membrane was incubated in to ECL Pus western blot detection reagent (ECL kit, Amersham Biosciences) for 5 min on a transparency sheet, in dark. PVDF membrane was
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placed in to the Hyper Cassette and superimposed with high performance chemiluminescence film in the dark room for 2 min and the protein signal was then developed on to the high performance chemiluminescence X-ray film by using developer and the signal was fixed by processing chemical fixer. The film was gently washed with tap water and dried. The density of the bands was arbitrarily quantified using Quantity One software of Bio-RAD gel documentation system (Bhushan et al., 2007).

3.0 STATISTICAL ANALYSIS

Data is expressed as mean ± SD, unless otherwise indicated. Comparisons were made between control and treated groups unless otherwise indicated using one way ANOVA by Sigmasat Software and p-values < 0.001 were considered significant.

4.0 RESULTS

4.1 Inhibition of cell proliferation by C-SLNs

Using a conventional tetrazolium-based colorimetric cell proliferation assay, we evaluated the cytotoxicity (Shashi et al., 2006) of C-SLNs in different cancer cell lines. Treatment of cancerous cells with curcumin and C-SLNs for 48 h produced concentration dependent inhibition of cell proliferation of HL-60, A549 and PC3. C-SLNs showed concentration dependent inhibition of cell growth with IC$_{50}$ of 1 pg/ml which is 85.7% less than the free curcumin (IC$_{50}$=7 µg/ml). Similar reductions in IC$_{50}$ were observed in other cell lines also. While IC$_{50}$ was 57% lower than free curcumin in case of A549 cells, 76% reduction was obtained in case of PC3 cell lines (Figure 1).

4.2 C-SLNs induce DNA fragmentation typical of apoptosis

To investigate whether treatment with C-SLNs induced higher DNA fragmentation than free curcumin, genomic DNA was isolated from treated HL-60 cells. Apoptosis typically involves intranucleosomal chromatin cleavage by endonucleases in multiples of 180 bp leading to DNA fragmentation resulting in a typical DNA laddering. Cells treated with C-SLNs for 18 h exhibited typical DNA ladder at both the selected concentrations (10 and 20 µg). However with free curcumin, at similar concentrations, DNA ladder was barely visible (Figure 2).
Figure 1. Influence of treatment with different concentrations of CSLNs curcumin on proliferation of various human cancer cell lines. The left side of the figure represents the treatment of cells with CSLNs while the right side of the figure represents treatment with free curcumin. Data are Mean ± SD (n = 8 wells) and representative of two similar experiments.
4.3 C-SLN increase Sub-G₀ DNA fraction of cell cycle phase distribution

HL-60 cells treated with C-SLNs for 24 h exhibited concentration dependent increase in sub-G₀ DNA fraction (≤2nDNA; Figure 3). The sub-G₀ fraction was 2% in control cells, which increased to ~96% after treatment with 10 μg/ml of C-SLNs while the sub-G₀ fraction for free curcumin was only ~54%, at 10 μg/ml (P values: ≤0.001 compared to untreated control). The sub-G₀ fraction comprises of both the apoptotic and debris fraction, implying the extent of death which has occurred. Results clearly indicated the potential of C-SLNs to increase the sub-G₀ fraction, as compared to free drug.
Figure 3. DNA Cell cycle analyses of HL-60 cells treated with C-SLNs at curcumin at 1 and 10 μg concentration for 24 h. Fraction of cells for hypodiploid (sub-G0, ≤2n DNA) population are from FL2-A vs. cell counts is shown (%). Data are representative of two similar experiments.

4.4 Loss of mitochondrial membrane potential by C-SLNs

HL-60 cells exposed to C-SLNs and free curcumin (1 and 10 μg/ml for 1 h) were analyzed for mitochondrial membrane potential (∆Ψm) loss employing Rh-123 by flow cytometry. In the untreated control cells, almost all cells were fluorescent with high Rh-123 fluorescence (Figure 4). C-SLNs at 10 μg/ml caused mitochondrial damage and hence the decrease of mitochondrial membrane potential by about 44% in comparison to free curcumin which showed only a 9% decrease at the same concentration (10 μg/ml). The loss of ∆Ψm is largely due to the opening of mitochondrial permeability transition pores (PTP), which conduit the leakage of cytochrome c and pro-apoptotic proteins from mitochondria to the cytosol. Significant loss in membrane potential, post treatment with SLNs, is a proof of its effectiveness to cause cell death.
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4.5 C-SLN stimulation of caspase activities in HL-60 cells

C-SLNs produced a dose dependent increase in the activities of caspase-8 and -9 in HL-60 cells (Figure 5). After incubation for 6 h with C-SLNs the effect was 3 times that achieved with free curcumin at the same dose (10 μg/ml). Furthermore, there was an even greater increase (4.4 times; p<0.001) of caspase 3 activity, postreatment with curcumin SLNs at 10 μg/ml in comparison to free curcumin.

4.6 Effect of C-SLNs on other apoptotic related genes expression

Treatment with C-SLNs at 10 and 20 μg/ml concentration also increased the release of cytochrome c into the cytoplasm of HL-60 cells (Figure 6). It is known that cytochrome c releases from mitochondria into the cytosol and binds to the apoptotic protease activating factor (Apaf) complex and triggers the activation of procaspases to the active caspase-9 (Reed, 1997). As shown in Figure 6, we observed significant increase (p<0.001) in the marked fraction of the cytochrome c release...
C-SLNs induced differential activation of various caspases: (a) caspase 3; (b) caspase 8; (c) caspase 9 in HL-60 cells. The cells in culture were exposed to 1 and 10 μg/ml of C-SLN free curcumin for indicated time periods. Data are Mean ± S.D of three similar experiments. Values at P: ≤0.001 were considered significantly different as compared to untreated control and curcumin treated cells.
Figure 6. Influence of curcumin and C-SLNs on the expression of important proteins involved in the initiation of apoptosis
HL-60 cells were treated with 30 μg/ml of TPD for indicated time periods in the presence and absence of NAC and sMIT. B-Actin was used as internal control to represent the same amount of proteins applied for SDS-PAGE. Specific antibodies were used for detection of cytochrome c, NF-κB, and TNF-R1. Data are representative of one of two similar experiments. P values < 0.001 were considered to be significantly different as compared to untreated control and curcumin treated cells.

5.0 DISCUSSION

In view of the enhanced bioavailability achieved with the developed C-SLNs (Kakkar et al., 2011b) we intended to confirm whether the developed system retains the anti-cancer potential of free curcumin or not. For this purpose the in vitro anticancer effect of curcumin and C-SLNs was evaluated in a variety of cancer cell lines. Hundreds of papers elaborate on the anticancer effects of curcumin at varying dose ranges (Chandel et al., 2006; Kunnumakkara et al., 2008; Mendonça et al., 2009; Shishodia et al., 2007a), but a relatively poor stability and poor bioavailability has been highlighted as one of the major problems in its therapeutic applications. Stability of curcumin in cell culture medium containing 10% FBS or in human blood is greater than in phosphate buffer but 50% of the molecule still decomposes within 8 h of incubation (Basile et al., 2009). Even though curcumin holds a multitargeting potential, however it cannot be translated into an efficient clinical accomplishment due to its compromised bioavailability. Later being assigned to its poor aqueous solubility, permeation and absorption across the gut along with a high rate of metabolic transformation (Anand et al., 2000; Lin et al., 1994).

Results of the present study clearly indicate the advantage of incorporating curcumin into SLNs. The extent of cell death induced by C-SLNs was significantly higher (p ≤ 0.001) at all the tested doses and in all the cell lines. Possible reasons include...
the better effect could be: (i) presentation of curcumin in a soluble form for interaction with the cancerous cells, (ii) better permeability of C-SLNs into the various cancerous cells, either because of a nanoparticle size which allows easy transport across the cellular membranes, or because of their composition. SLNs constitute a lipid and surfactants including phospholipids. Latter have an intrinsic nature to intermingle with cellular membranes. Further presence of other surfactants can also enhance the permeability characteristics of SLNs (iii) stability of curcumin is ensured when incorporated into SLNs.

A study report on polymeric nanoparticles of curcumin demonstrated that nanocurcumin formulation has comparable efficacy to free curcumin against pancreatic cancer cell lines in vitro (Bisht et al., 2007). Further, they confirmed that nanocurcumin retains the mechanistic specificity of free curcumin, inhibiting the activation of the seminal transcription factor NF-κB, and reducing steady state levels of pro-inflammatory cytokines like interleukins and TNF-α (Bisht et al., 2007). However, the authors do not report on the molecular cascade of events associated with the induction of apoptosis in these cancer cell lines. In another study, a liposomal curcumin formulation also demonstrated a comparable potency to free curcumin (Li et al., 2005). Even as further studies with this liposomal formulation are awaited, it is emphasized that liposomes, which are metastable aggregates of lipids, tend to be more heterogeneous, and larger in size (typically 100–200 nm) than most nanoparticles.

The results of the present study describe the significantly higher apoptotic activity of developed C-SLNs on human leukemia HL-60 cells, for the first time. This is evidenced from the low IC₅₀ values achieved with C-SLNs as compared to free curcumin in MTT test in all the cell lines and especially in HL-60 cells. Further, measurement of DNA fragmentation, indicated that the cancer cell death is due to the induction of apoptosis by C-SLNs on HL-60 cells while no such fragmentation was observed with free curcumin at the same concentration. It may be highlighted here that previous studies (Bisht et al., 2007; Li et al., 2007a) report on only a similar effect being achieved with a modified/novel delivery system of curcumin while we report on a several times enhanced effect being achieved with C-SLNs.

Inhibition of dysregulated cell cycle progression in cancer cells is an effective strategy to halt tumor growth (Singh et al., 2002). Our results demonstrate that
treatment of HL-60 cells with C-SLNs induces sub-G₀ phase arrest of cell cycle progression indicating it to be one of the mechanisms by which they inhibit proliferation. Additionally C-SLNs remarkably increased caspase-9 activity; activation of latter being solely regulated by mitochondrial release of apoptotic protease activating factor-1 (Apaf-1) and cytochrome c, which is a part of mitochondrial dependant apoptosis pathway (Debatin, 2004). Further, caspase-8 activation in HL-60 cells was also induced probably because of the upstream over-expression of cell surface receptors such as TNF-R1 and suppression of NF-κB (Liao et al., 2008). Various study reports enumerate the effect of curcumin induced apoptosis in human melanoma cells (30–60 μM for 24 h) (Bush et al., 2001), human leukemia HL-60 cells (10–40 μM for 16–24 h) (Anto et al., 2002; Kuo et al., 1996) apart from AK-5 tumor cells and MCF-7 breast cancer cells (Bhaumik et al., 1999; Choudhuri et al., 2002; Khar et al., 1999) but no such report exists wherein effect of the curcumin loaded drug delivery system has been established to have a prospective apoptotic effect as compared to free drug.

From the above it can be said that there are varying mechanisms responsible for apoptosis induced by curcumin. Latter include Akt dephosphorylation; Bcl-2, Bcl-XL, and inhibitor of apoptosis protein inhibition; cytochrome c release; and caspase-3 activation (Khar et al., 1999).

To verify the hypothesis that the mitochondrial DNA damage induced by curcumin in HL-60 cells may be the event triggering apoptosis by C-SLNs, we analyzed the ΔΨₘ during the time-course experiments by using rhodamine 123. Interestingly, after an incubation of 6 h, hyperpolarisation of mitochondrial membrane was observed with C-SLNs treatment which was significantly more (p≤0.001) than free curcumin. Hyperpolarisation seems to represent a prerequisite for rapid mitochondrial-mediated apoptotic cell death that eventually leads to the loss of mitochondrial membrane potential (Cao et al., 2007).

Cytochrome c seems to be a critical factor in apoptotic process (Reed, 1997). Cytochrome c released from the mitochondria into the cytosol forms a complex with the apoptosis-activating factor-1 and procaspase-9, which results in the maturation of caspase-9 and subsequently activates caspase-3 (Woo et al., 2003). Our finding of C-SLNs induced translocation of cytochrome c from the mitochondria to the cytosol provides a direct link between the mitochondria and the bioenhanced C-
SLNs induced apoptosis in HL-60 cells. The release of cytochrome c in apoptosis is controlled by Bcl-2 family members (Kuo et al., 1996). Bcl-2 family proteins are important regulators of apoptotic signaling, acting to either inhibit or promote cell death. The Bcl-2 protein is known to inhibit apoptosis by regulating ΔΨm and cytochrome c release is needed for activation of caspase-9. Our results promisingly indicate the low dose apoptotic potential of C-SLNs to fight against the deadly cancer era which is metastasizing to millions of people around the globe.

6.0 CONCLUSION

The usefulness of natural molecules like curcumin is limited in therapy because of their poor physicochemical characteristics. Curcumin is a multitargeted molecule showing pluripharmacology such that it manifests several anticancer mechanisms complementing the overall effects. In spite of wondrous in vitro success, data on curcumin and its clinical effectiveness are still lacking. The ambiguity in translating in vitro and preclinical effectiveness to humans and clinics points towards a need for pharmaceutical couturing of curcumin. The significance of this statement can be understood from highlighted apoptotic role of C-SLNs as compared to free curcumin, against a variety of cancer cell lines, presented herein. Most of the investigations report on pharmacological and clinical studies showing effectiveness of curcumin for gut-related cancers only. This is as expected, considering that curcumin is a very poorly absorbed drug (less than 1%) and is excreted unchanged in the faeces. Hence its local concentration in the gut is sufficient to show this effect. Similarly, it has also shown its clinical effectiveness upon local application to skin.

In view of the above, we present a solid lipidic nanoparticulate dispersion of curcumin with enhanced bioavailability (32-155 times) as discussed earlier (Kakkar et al., 2011b). Present investigation deliberately deals with a proof of concept of in vitro efficacy of developed C-SLNs over free curcumin.

Having achieved the in vitro efficacy and enhanced in vivo systemic bioavailability, we expect the formulation to effectively hit the target for this most appalling disease i.e cancer. However, future studies on developed formulation will enable addressing these scenarios in an in vivo setting, and should facilitate the eventual clinical translation of this well known herbal agent to the status of a therapeutic agent.