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Curcumin in chronic lymphocytic leukemia – A review

Vagish Kumar Laxman Shanbhag*

Department of Oral Medicine and Radiology, Yenepoya Dental College and Hospital, Yenepoya Research Centre, Yenepoya University, Mangalore 575018, Karnataka, India

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ABSTRACT

Curcumin is a widely researched natural product and is known to possess anti-carcinogenic properties. Chronic lymphocytic leukemia is a type of leukemia that principally affects patients with age higher than 60 years. Since the toxicity of conventional drugs exceeds the benefits of treating this leukemia type, patients are treated only in the advanced symptomatic stages. The current article reviews curcumin, its general actions and targets in cancer, and specifically that of it in chronic lymphocytic leukemia.

1. Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia in western countries [1,2]. It occurs predominantly in males than females [1]. The elderly population is commonly affected with a median age of diagnosis at 70 years [3]. The disease is frequently detected in routine blood check-up [3]. CLL is now-a-day detected in younger people also due to the availability of frequent blood check-up [1]. The disease is most commonly asymptomatic and runs a highly variable clinical course [1]. CLL is characterized by the accumulation of mature-appearing lymphocytes, particularly CD-5 positive B-Cells in the blood, bone marrow, spleen and lymph nodes [1,3]. CLL originates from mature B-lymphocyte progenitor [4]. In CLL, the B-cells have long lifespan leading to their abnormal increased accumulation in the body [4]. CLL cells express CD40 which regulates differentiation, proliferation, and survival of B-cells [5]. Nuclear translocation and activation of NF-kappa B require CD40 ligation in normal cells. It is found that CD40 ligation promotes NF-kappa B activity in CLL B-cells [5]. The interaction between CD40 and CD154 is observed to be necessary for a subset of CLL B-cell survival [5]. In most of the CLL cases, NF-kappa B activity has been observed to be up-regulated [5]. In these patients, T-cell and natural killer cell count are also observed to be raised and this is correlated to delayed disease progression [4]. The disease is caused by alterations in micro-RNA genes of B-lymphocytes resulting in increased resistance of B-Cells to apoptosis culminating in leukemic transformation [1]. CLL can be diagnosed positively by detecting the presence of $\geq 5 \times 10^9$/L monoclonal cells that co-express CD5 and CD23 [1,3]. The B-Cells may also co-express CD19, CD40, and CD154 [5]. Clinical features range from lymphadenopathy, splenomegaly, hepatomegaly, anemia and thrombocytopenia [1,3]. CLL patients may present impaired antibody production which may culminate in a pan- or partial hypogammaglobulinemia [6]. CLL may transform to Hodgkin lymphoma, non-Hodgkin's lymphoma, prolymphocytic leukemia, multiple myeloma, hairy cell leukemia and acute leukemia [7]. Conventional allopathic treatment is indicated

*Corresponding author: Dr. Vagish Kumar Laxman Shanbhag, Reader, Department of Oral Medicine and Radiology, Yenepoya Dental College and Hospital, Yenepoya Research Centre, Yenepoya University, Mangalore 575018, Karnataka, India.
Tel: +91 8147810947
E-mail: vagishkumar_12@rediffmail.com.

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only in the advanced stage of disease because of toxicity of these drugs [2,8]. CLL is incurable with current treatment modalities [9]. Patients are usually treated with fludarabine, cyclophosphamide, rituximab, obinutuzumab, chlorambucil, alemtuzumab, bendamustine, lenalidomide and ibrutinib [1,2,10,11]. Because of the increasingly adverse effects of the drugs used for managing CLL, alternative mode of its management using natural products has been researched. Some of the natural products researched are epigallocatechin gallate, curcumin, resveratrol, lentinan, genistein, phenethyl isothiocyanate (PEITC) and citrus pectin-derived galectin-3 inhibitor GCS-100 [11,12]. Among these natural products, curcumin is the most researched one [13].

2. Curcumin and its mechanism of action in cancer

Curcumin, a hydrophobic natural polyphenol is derived from the rhizome of turmeric (*Curcuma longa*) [13,14]. Turmeric is a tropical plant originating from Southern and Southeast Asia [4]. It is well known in Chinese medicine for its use in preventing and treating various human diseases [14]. Turmeric is known by various names such as zirsood, holdi, haridra, manjaland Indian saffron, and haldi [14]. It is the key ingredient of curry in sub-continental cooking [14]. Turmeric powder is yellow colored and contains various curcuminoids such as curcumin (77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3%) [14,15]. Curcumin, a diferuloylmethane is the main curcuminoid in turmeric [13]. Curcumin is traditionally used in the treatment of various diseases such as sinusitis, allergy, asthma, cough, hepatic disease, coryza and bronchial hyperactivity [14]. It is used in wound healing and to prevent renal failure in diabetes, Alzheimer's disease, and heart disease [14]. Curcuminoids are derived from turmeric by ethanol extraction [16]. Curcumin has strong anti-oxidative, chemopreventive, chemotherapeutic, chemosensitizing and anti-inflammatory properties [9,17]. It also has anti-infectious, anti-arthritic, hepatoprotective, thrombosupressive, and anti-carcinogenic properties [14]. Curcumin is also known to boost the body's immune system [9]. Tumor initiation, promotion, progression, and metastasis are observed to be suppressed by curcumin [4,9]. Curcumin inhibits cellular proliferation, invasion, and metastasis by suppressing multiple signaling pathways [18]. Curcumin is lipid soluble and has anti-HIV and anti-diabetic activities [19]. Curcumin possesses anticarcinogenic activity because of its antioxidant and pro-oxidant properties [19]. Curcumin inhibits free radicals from causing peroxidation of lipid membranes and oxidative DNA damage [19]. Phenolic group of curcumin is responsible for its antioxidative properties [19]. Curcumin is demonstrated to possess pro-oxidant properties [19]. Turmeric is readily available and is generally recognized as safe in the United States by Food and Drug Administration (FDA) [14].

Cancer is proposed to be caused by defective inflammatory pathway [14]. Chronic inflammation increases the formation of pro-inflammatory molecules like Reactive oxygen species (ROS), cytokines, cyclooxygenase (COX-2), oncogenes, intracellular signalling pathway mediators, transcription factors such as activator protein 1 (AP1), nuclear factor κB (NF-κB), protein kinases B (AKT), signal transducer and activator of transcription 3 (STAT3) [14]. Curcumin inhibits multiple cellular and molecular targets and pathways such as inflammation (NF-κB, tumor necrosis factor, interleukin-1, COX-2, and 5-Lipoxygenase), Tumor necrosis factor alpha (TNF-α), interleukin-6 induced STAT3 phosphorylation (Signal Transducer and Activator of Transcription), proliferation (AP-1, human epidermal growth factor receptor-2, epidermal growth factor receptor), cell cycle (cyclin-D1 and cyclin E), apoptosis (activation of caspases and down-regulation of anti-apoptotic gene products), survival (phosphatidylinositol-3 kinase/protein kinase B pathway), invasion (matrix metallopeptidase-9 and adhesion molecules), angiogenesis (vascular endothelial growth factor), metastasis (C-X-C chemokine receptor type 4) and oncogenic kinases such as extracellular signal–regulated kinases 1/2, mitogen-activated protein kinases (p38 mitogen-activated protein kinase, c-Jun N-terminal kinases 1/2) [13,14]. Curcumin also influences the action of activation of transcription factors (*e.g.*, nuclear factor E2-related factor 2, peroxisome proliferator-activated receptor protein- γ, and hypoxia-inducible factor -1), receptors (*e.g.*, HER-2 and IL-8), kinases (*e.g.*, EGFR, Janus Kinase, and ABA-activated protein kinase), cytokines (*e.g.*, macrophage inflammatory proteins, and monocyte chemoattractant protein), enzymes (*e.g.*, inducible nitric oxide synthase, Glutathione S-transferase, and ATPase, histone deacetylase, DNA methyltransferase and histone acetyltransferase), and growth factors (*e.g.*, epidermal growth factor, nerve growth factor, hepatocyte growth factor and platelet derived growth factor) [13,20]. Curcumin inhibits anti-apoptotic proteins-induced myeloid leukemia cell differentiation protein (Mcl-1) and X-linked inhibitor of apoptosis protein (XIAP), and upregulates the pro-apoptotic protein Bcl-2-like protein 11 (BIM) [4]. Notch-1 signaling, early growth response-1 gene product (Egr-1), farnesyl-protein transferase (FPTase), telomerase, c-Myc, fibroblast growth factors (FGF) mediated cell signaling are also known to be inhibited by curcumin [18,21]. Cellular signaling pathway of TNF-related apoptosis-inducing ligand is known to be modulated by curcumin [20].

Curcumin contributes to anticancer activity by negatively regulating transcription factors, inflammatory cytokines, growth factors, oncogenes, reactive oxygen species (ROS) and protein kinases [14,15]. Curcumin activates peroxisome proliferator-associated receptor gamma (PPAR-γ), forkhead box O3F (FOXO3a) and p53 [14]. Curcumin upregulates JNK, death receptor-4, death receptor-5, differentially expressed in FDCP (DEF-40), Nrf-2, and estrogen responsive element (ERE) [13,15,18]. In leukemia, curcumin is observed to inhibit TNF-α mRNA in K562 cell lines [14,15]. Curcumin induces the formation of glutathione-S-transferase (GST), hemoxygenase-1 and quinine reductase which neutralize the formation of ROS [14]. STAT-3 is involved in leukemia and curcumin inhibits STAT-3 [14]. Curcumin suppresses prostrate-specific antigen [17].

Reactive oxygen species (ROS) such as superoxide, hydroxyl, peroxyl radicals, hydrogen peroxide; and reactive nitrogen species (RNS) such as nitric oxide cause oxidative damage to cell DNA, proteins, lipids resulting in carcinogenesis and development of leukemia [19]. Enzymatic oxidants like superoxide dismutase, catalase, and glutathione peroxidase; and nonenzymatic antioxidants such as glutathione, thioredoxin, ascorbic acid in the body neutralize the ROS, RNS and H_2O_2 [19]. Imbalance in the homeostasis between free radicals and antioxidant defense system of cells is the cause of leukemia [19]. Leukemic cells produce more free radicals than normal counterpart [19]. Superoxide dismutase and catalase activity are observed to progressively decrease in

chronic lymphocytic leukemia patients who are not on chemotherapy resulting in increased levels of oxidation products 8-oxo-dG and malondialdehyde (MDA) which are indicators of accumulation of oxidative damage to DNA and lipids [19]. Oxidative DNA damage modifies the regulation of transcription, induces replication errors leading to genomic instability or deviation of signaling pathways [19]. Chronic inflammation induces accumulation of ROS, resulting in damage to DNA and lipids and ultimately to cancer development [19]. Since cellular antioxidant capacity is decreased in leukemia, administration of antioxidants like curcumin is highly beneficial [19]. Curcumin promotes apoptosis in several cancer cell lines and enhances the anti-cancer effects of chemotherapy [17].

Curcumin neutralizes cancer cells by inducing apoptosis and/or arresting them at different cell cycle phases at G0/G1 and/or G2/M phase [15,19]. It interacts with multiple intracellular and extracellular molecules that have a role in cancer initiation and progression [15]. Curcumin suppresses Wnt/β-catenin pathway [15]. Curcumin enhances the formation of Nrf-2 which has antioxidant and detoxifying activities [15]. Curcumin inhibits inducible nitric oxide synthase and lipoxygenase [15]. Curcumin inhibits JAK-STAT3 phosphorylation in K562 chronic leukemia cells through suppression of JAK2, cyclin D1, and v-src gene expression. In chronic lymphocytic leukemia, curcumin downregulates JAK-STAT3 pathway [15].

Surprisingly curcumin has prooxidant activity which can have anticancer effects through ROS generation [19]. It is observed that in HL-60 human leukemia cells, low doses of curcumin (<20 μM) decrease ROS production, whereas higher concentrations, increase ROS production [19]. The increased ROS generated because of higher curcumin concentration has a killer effect on cancer cells without much affecting the normal cells [19]. ROS generated with high concentrations of curcumin is observed to inhibit histone acetyltransferase which is required for histone acetylation [19]. Histone acetylation opens the chromatin structure of DNA, making transcription factors required for activating genes of cell proliferation become susceptible to free radicals [19]. Ascorbic acid and glutathione augment the activities of low dose curcumin [19]. Thus these water-soluble antioxidants in combination with curcumin should be a part of the regimen of an effective anti-cancer therapy [19]. Curcumin has the ability to transform thioredoxin reductase into a pro-oxidant. The resultant increased ROS levels damages DNA and hampers the NF-κB modulated cancer survival mechanisms [19]. Curcumin also increases glutathione efflux resulting in antitumor activity through initiating apoptosis [19]. Curcumin inhibits kinase Jak 1 and its influence of STAT3 resulting in growth arrest and apoptosis in leukemia [19]. Curcumin inhibits NF-κB signal transduction resulting in decreased γ-glutamyltransferase (GGT) [19]. Increased production of GGT is known to cause drug resistance in cancer and also in inflammatory leukotriene synthesis [19]. Curcumin upregulates 70 kDa heat shock proteins (Hsp70), which in turn inhibits NF-κB activation [19]. CLL cells become drug resistant due to stromal protection [19]. This stromal protection can be prevented by using high dose curcumin [19]. Epigallocatechin-3 gallate has additive effects with curcumin by inducing apoptosis in CLL. The addition of epigallocatechin-3 gallate makes possible even the low dose of curcumin to effectively block stromal protection [19]. Curcumin, when used with mitomycin C, protects human lymphocytes from genotoxic effects [19]. In addition, patients with CLL benefit from curcumin due to its immunopotentiator properties resulting in protection from infectious diseases[CLL] [19]. Low doses of curcumin enhance body's antibody response [16]. Curcumin together with vitamin D3 effectively exerts an anti-leukemic effect by inhibiting NF-kappa B [22]. Curcumin is also known to promote mitochondrial membrane potential loss in leukemia [20].

Curcumin in addition to interrupting cell cycle also disrupts mitotic spindle structures, induces micronucleation and apoptosis and thereby has antiproliferative activity [16]. Curcumin, by inhibiting IL-2 gene expression and suppressing NF-kappa B, has antiproliferative activity in T-cell leukemia [16]. DNA methyltransferases promote methylation of promoter CpG of tumor-suppressor genes leading to blood cancer. Curcumin is discovered to induce global DNA hypomethylation in a leukemia cell line [23].

Leukemia stem cells may lead to leukemic relapse, drug resistance, and mortality even after hematopoietic stem cell transplantation. Five researchers in their study observed that curcumin, when administered together with busulfan, induces inhibition of cell growth and enhances apoptosis in busulfan resistant KG1a leukemic stem cells. Curcumin plus busulfan upregulated Bcl-2-associated death promoter (BAD), caspase-3, and proapoptotic serine protease HTRA-2. This drug combination downregulated B-cell lymphoma 2 (Bcl-2), cellular inhibitor of apoptosis-2 (cIAP-2), survivin, and X-linked inhibitor of apoptosis (XIAP). Curcumin also was synergistic to the cytotoxic effects of busulphan. This action was attributed to the downregulation of survivin expression. Also, it was observed that cells at G0/G1 phase were more sensitive to this combination drug therapy. The addition of curcumin also enables the dose reduction of busulfan. Curcumin is known to potentiate the anti-apoptotic actions of bortezomib, paclitaxel, 5-fluorouracil and folfox both *in vitro* or *in vivo* [24].

A study observed that resistance of leukemic stem-like KG1a cells to arsenic trioxide can be overcome by addition of curcumin. They attributed this action to down-regulation of survivin expression. The study detected enhanced apoptosis, indicated by increased levels of Annexin V/Propidium iodide. Also, cytotoxicity due to growth inhibition was increased. Curcumin, enhanced arsenic trioxide-induced apoptosis, by inducing caspase-3 activation and PARP cleavage. Thus curcumin represents an excellent chemosensitizing agent in the management of leukemia [25].

Curcumin is selectively toxic to cancer cells than normal cells and enhances the cytotoxic activity of chemotherapeutic agents. Selective actions of curcumin are due to differences in its metabolism between normal and cancer cells. Proto-oncogene mutations in cancer cells cause increased levels of free radicals. Curcumin selectively acts as pro-oxidant in cancer cells with increased free radical levels, resulting in their apoptosis. Curcumin is also known to inhibit anti-apoptotic genes- for example- survivin, which is overtly expressed in leukemic cells. This overexpression of anti-apoptotic genes is a known cause for increased drug resistance to chemotherapeutic agents. Etoposide is used in the treatment of leukemia. It is known to upregulate NF-kappa B. Curcumin is known to inhibit NF-kappa B activation, nuclear translocation and its binding to DNA, thus promoting cytotoxicity in cancer cells by increasing apoptosis and genotoxicity, when administered with etoposide. Etoposide has several side effects on bone marrow cells. Curcumin is one of

the natural medicines which can potentiate the cytotoxic action of etoposide on cancer cells without increasing side effects on normal cells. Papież *et al*. in their study observed that 20 μM curcumin, when combined with 10 μM etoposide, increased oxidative stress in HL-60 cells indicated by decreased levels of glutathione and increased free radical levels, resulting in cytotoxicity and cell growth arrest. The study also noted that administering/pretreatment curcumin at a dose of 200 mg/kg enhanced the anti-leukemic activity of etoposide in the rat model of acute myeloid leukemia. Curcumin, enhanced etoposide-induced apoptosis, in a selective way in this rat model. No inductions of apoptosis of non-leukemic cells were observed. The study attributed the cytotoxic actions of curcumin to oxidative stress. Curcumin was observed to protect normal marrow cells from cytotoxic effects of etoposide [26].

Bortezomib and curcumin inhibit NF-kappa B and have activity against chronic lymphocytic leukemia. Nagy *et al*., in their study, developed a novel mannich-type curcumin derivative C-150 with metahydroxyphenyl side-chains and 3-phenyl-3-acrylamido branched central motif. They observed that both curcumin and its analogue C-150 inhibited NF-kappa B activation and had additive effects with bortezomib by inducing apoptosis in HL-60 cells. Similar results were obtained in HL-60 xenograft SCID mice. Also found was that concentration of C-150 required for inducing apoptosis was 50 fold lesser than that of curcumin. Curcumin, bortezomib, and their combination, negatively regulate JNK signaling in CLL [20].

Hassan *et al*. studied the metabolism, cytotoxicity and epigenetic changes caused by curcumin and dimethoxycurcumin (DMC) in leukemic cells [27]. In DMC, the two phenolic hydroxyl groups of curcumin are replaced by methoxy groups [27]. Clinically relevant achievable concentrations of curcumin in plasma are 1.77 ± 1.87 μmol/L [27]. The study found that though curcumin and DMC were not cytotoxic to leukemic cells at clinically relevant concentrations (1 μM and 2 μM), they induced cell cycle arrest at G2/M phase. The study also observed that DMC induced p15 and CDH-1 promoter-methylated gene expression without the reversal of DNA methylation, whereas curcumin did not show this action [27]. The authors discovered that DMC is more stable metabolically, has higher bioavailability and cytotoxic to leukemia cells than curcumin in clinically relevant concentrations [27]. Curcumin in 2 μM concentration induced extensive apoptosis in BV-173 cells when treated for 168 h. However, DMC was more potent than curcumin in inducing apoptosis. The authors suggested that combination of DMC and DNA methyltransferase inhibitors could be synergistic and useful to leukemia patients [27].

Kuo *et al*. in their study, observed that curcumin was capable of inducing apoptosis in promyelocytic leukemia HL-60 cells at low concentrations of 3.5 μg/ml. Chromatin DNA fragmentation was noticed in cells after 4 h of exposing them to curcumin. At concentrations of 14 μg/ml, curcumin caused necrosis of cells. The authors attributed the apoptosis caused by curcumin to Bcl-2 proteins [28]. Curcumin inhibits the expression of Bcl-2 at post-translational level leading to decreased levels of Bcl-2 proteins. A raised level of Bcl-2 is known to protect cells from undergoing apoptotic cell death [28].

Ceramide is observed to get accumulated in cells when sphingomyelin synthases (SMS) and glucosylceramide synthase (GCS) get downregulated, resulting in cell apoptosis. Thus ceramide has pro-apoptotic activity. Tumor cells exhibiting multidrug resistance are noted to exhibit increased activity of SMS and GCS. Also, expression of P-glycoprotein (P-gp) is upregulated in these multidrug resistant tumor cells, resulting in expelling of cancer drugs out of the cell. This multidrug resistance leads to failure of cancer chemotherapy. Shakor *et al*. in their study observed that curcumin instigates apoptosis in both drug-sensitive human leukemia HL60 cells and in HL60/VCR multidrug resistant leukemic cells exposed to vincristine. Curcumin activates neutral sphingomyelinase (nSMase) through glutathione depletion and later inhibits SMS through caspases, particularly caspase-3, resulting in ceramide build-up in cells, which eventually culminates in the instigation of apoptosis. In the case of HL60/VCR cells only, curcumin additionally inhibits GCS also by inhibiting/downregulating P-gp [29].

Gao *et al*. in their study noticed that pure curcumin downregulates Wilms' tumor 1 (WT1) gene expression in leukemic cells (K562 and HL-60), partly by upregulating miR-15a/16-1 expression [30]. Raised WT1 levels (1000–10 000 folds) are observed in primary leukemic cells, such as that of acute leukemia and chronic myelogenous leukemia, than their normal counterparts. Downregulation of WT1 induces apoptosis and prevents the proliferation of HL-60 and K562 cells [30].

Three authors in their study investigated the growth inhibiting actions of curcumin on acute promyelocytic leukemia (APL) cells [31]. They observed that curcumin stimulates apoptosis and inhibits growth in APL cells. Curcumin sensitizes APL cells by stimulating unfolded protein response (UPR)-induced apoptosis by enhancing build-up of atypically phosphorylated misfolded nuclear receptor corepressor (N-CoR) protein in the endoplasmic reticulum (ER), by impeding its endoplasmic reticulum-associated degradation (ERAD), proteasomal-mediated degradation, and protease-mediated degradation [31].

Five authors studied the combinational effect of CdSe/ZnS quantum dots (QDs) and/or curcumin, with or without ultraviolet A (UVA) irradiation on HL-60 leukemia cells and normal lymphocytes [32]. The authors found that curcumin and UVA irradiation enhanced apoptosis, cell death, ROS generation, and single/double DNA strand breaks induced by QDs in HL-60 cells. In contrast, curcumin had antioxidant/protective effects against QD treatment under UVA irradiation induced ROS generation, cell viability and apoptosis in human normal lymphocytes. However, there was no protective effect of curcumin against QD-induced single DNA strand breaks in normal lymphocytes. The authors conveyed that curcumin's cytotoxicity or genotoxicity in leukemia cells is through decreased glutathione levels brought about by ROS generation, activated caspase pathway, arrest of G2-M phase in cell proliferation pathway, increased uptake of curcumin in leukemic cells when compared to normal lymphocytes and downregulation of cyclin D. The study suggested that combination of CdSe/ZnS QDs with curcumin presents a worth exploring area in treatment of leukemia [32].

A group of authors observed that curcumin induces DNA topoisomerases (topos) I and II-DNA complexes in K562 leukemia cells [33]. The formations of these complexes are mediated by reactive oxygen species. These complexes are then converted to permanent DNA strand breaks by cellular processing that eventually results in cell death. Curcumin is capable of inducing 8-Oxoguanosine (8-oxoG) in both cell systems and in isolated DNA. 8-oxoG enhances topo I binding to DNA and raises DNA complex formation by three to seven times. The

authors observed that the levels of these complexes induced by curcumin were much higher than that induced by equitoxic doses of several standard DNA topoisomerases I and II inhibitors such as camptothecin and etoposide. However, the authors cautioned that high oral doses of curcumin can cause carcinogenic effects due to the formation of nonlethal levels of topo-DNA complexes [33].

Chen *et al*. in their study discovered that trichostatin A improves the anticancer activity of low curcumin levels in HL-60 leukemia cells [34]. Trichostatin A (TSA) is a specific histone deacetylase inhibitor. The authors observed that curcumin levels less than 20 μM declined ROS generation, while high curcumin levels (50 μM and 100 μM) stimulated it. Also noted in this study, was that low curcumin levels did not have any influence on histone acetylation, whereas Trichostatin A increased histone acetylation. However, TSA did not have any influence on ROS generation. The study discovered that a low curcumin level when combined with TSA, has the advantage of both increasing histone acetylation and decreasing ROS accumulation. Thus this combinational therapy exhibited high toxicity to HL-60 cells. Histone hypoacetylation is known to favor tumor cells by preventing initiation of transcription of genes responsible for apoptosis and differentiation such as p53, adenomatous polyposis coli, K-ras and p14(ARF). The authors suggested that since high curcumin levels may increase ROS generation, its use may harm normal cells. So it would be advantageous and judicious to use low curcumin level therapy combined with TSA for better and safe anti-leukemic activity [34].

A group of authors in their study, administered 15 g/day turmeric for six weeks together with imatinib to 50 patients with chronic myeloid leukemia. They observed that the patients receiving turmeric along with imatinib exhibited a significantly higher reduction in nitric oxide levels when compared with the group receiving imatinib therapy alone. Nitric acid contributes to the development of cancer by causing DNA damage through various mechanisms involving transcription factors and protein kinases. The study concluded that curcumin may be used as an adjuvant to imatinib therapy to reduce nitric oxide levels in chronic myeloid leukemia patients [35].

Wu *et al*. developed a novel curcumin derivative C817 and studied its effects on wild-type (WT) and imatinib-resistant mutant Abl kinases. They also studied the actions of C817 cells on imatinib-sensitive and resistant chronic myeloid leukemia (CML) cells *in vitro*. The authors of the study discovered that C817 inhibited the activities of both WT and mutant Ab1 kinases. Also, C817 inhibited the growth and caused apoptosis of both imatinib-sensitive and resistant CML cells, in addition to potently inhibiting leukemia progenitor/stem cells. Apoptosis was through stimulating mitochondrial pathway. In this study, imatinib could not inhibit the growth of leukemia progenitor/stem cells. The study concluded that C817 is useful in treating CML patients exhibiting imatinib resistance due to Bcr-Abl kinase domain mutations [36].

3. Curcumin in chronic lymphocytic leukemia

The high B-cell count in CLL is due to accumulation of B-cells due to their decreased apoptosis rather than increased proliferation. Also, B-cells in CLL are non-cyclic. The cells have increased levels of anti-apoptotic proteins Bcl-2, Mcl-1, and XIAP (X-linked inhibitor of apoptosis protein). B-cells in CLL promote STAT3 pathway, VEGF signaling, activated NF-kappa B, and phosphatidylinositide 3-kinase (PI3K). Selective inhibition of PI3K induces apoptosis in CLL B-cells. Survival of CLL B-cells is promoted by physical contact of these cells with the stromal tissues in marrow cavity and also by the stromal cells released soluble factors. This action is facilitated by various integrins of CLL B-cells such as very late antigen-4 (VLA-4) with various marrow stromal cells ligands such as vascular cell adhesion molecule 1 (VCAM-1), fibronectin and iC3b. The protection offered by marrow stromal microenvironment is essential for the survival, proliferation, and upregulation of antiapoptotic proteins of CLL B-cells. Four authors in their study evaluated the cytotoxic effects of curcumin on CLL B-cells with or without the presence of marrow stromal cells. They observed that curcumin inhibits pro-survival pathways NF-κB, STAT3, and AKT. Combinative actions of curcumin and epigallocatechin-3 gallate (EGCG) on the stromal protection of CLL-B cells were also examined. EGCG inhibits VEGF receptor activation and increases apoptosis in CLL B-cells. Curcumin caused apoptosis in CLL B-cells with PARP cleavage in a dose-dependent fashion. The study suggested that curcumin induced apoptosis is not dependent on caspase pathway. They also found that anti-apoptotic protein XIAP and myeloid cell leukemia-1(Mcl-1) expression CLL B-cell was reduced by curcumin. Curcumin also increased pro-apoptotic BIM expression and inhibited AKT phosphorylation. The study also found that curcumin overcomes stromal protection of CLL B-cells when administered at high dose (20 μM). EGCG and curcumin, when administered sequentially increased apoptosis of CLL-B cells and had additive effects when compared to either drug used alone. Also, sequential administration of curcumin and EGCG overcame stromal protection with low doses of curcumin. It was also noted that EGCG followed by curcumin was more effective than curcumin followed by EGCG. The study suggested that EGCG and curcumin, when used simultaneously are antagonistic and have less than additive effects. In this study, curcumin did not have any effect on Bcl-2 or survivin [21].

Curcumin inhibits aminopeptidase N/CD13 (APN; gp150) which is expressed in considerable proportions of cultured mononuclear cells of CLL patients. A study confirmed concentration dependent cytoreduction and induction of apoptosis by curcumin in cultured mononuclear cells of chronic lymphocytic leukemia patients, immunolabelled by 7H5 and in all B-cell leukemia cell lines MEC-1, JVM-3, and BV-173 [9]. The study also observed the excellent response to curcumin in CD 13 positive cases [9]. APN production is observed to be increased in tumors and is considered to be of poor prognosis if detected in lymphoid malignancies [9]. APN promotes tumor angiogenesis, growth, invasion and metastasis [9]. The anti-leukemic activity of curcumin was attributed to its modulation of signal transduction such as inhibition of prosurvival factors – NF-kappa B, STAT3, Akt, spleen tyrosine kinase (SYK), and activation-induced cytosine deaminase (AID). SYK is noted to be overexpressed in CLL [34]. Also, phosphorylation of SYK is observed to be doubled in CLL than normal B-cells [34]. SYK is a key component of B-cell receptor signaling pathway. B-cell receptor signaling pathway plays an important role in conferring apoptosis resistance to CLL cells. SYK inhibitors are noticed to instigate apoptosis in CLL cells [37]. In addition to inducing apoptosis, hallmarks of curcumin include the release of cytochrome C from mitochondrial membrane and poly(ADP-ribose) polymerase cleavage in primary B-CLL

cells. The study concluded that CD13/APN inhibiting property of curcumin confers a new therapeutic target in the treatment of CLL [9].

The addition of arabinoxylan to curcumin therapy is shown to be beneficial to early stage CLL patients. Arabinoxylan possesses anti-inflammatory, pro-apoptotic and immunostimulatory effects [6]. A group of researchers observed 20% decreased absolute lymphocyte count in 22% CLL patients of the study when arabinoxylan was added to curcumin [6]. Also, CD4 and CD8 T-lymphocyte percentages increased in these patients [6].

Increased T cell and NK cells in CLL have been related to delayed disease progression. Five researchers studied the effect of 2 g/day oral curcumin in divided doses in the form of Meriva-natural curcuminoids and lecithin in 1:2 ratios on absolute lymphocyte count (ALC) and its effect on CD4, CD8, and NK cells count on twenty-one stage 0/1 CLL patients. Meriva also contains microcrystalline cellulose and has increased bioavailability and absorption. Curcumin increased CD4 T cell, CD8 T cell, and NK cell count through its immunomodulation action. The study observed decreased ALC and attributed this phenomenon to increased T cells due to curcumin administration. The study suggested that increasing the counts of anti-tumor T cells and NK cells through curcumin could be helpful in CLL patients. The study concluded that curcumin could be beneficial to a small subset of CLL patients. The limitation of the study was that it was carried on small patient sample [38].

Curcumin causes cytochrome c release from the mitochondrial membrane which may contribute to apoptosis of CLL B-cells [12]. Curcumin is known to potentiate the actions of vincristine on CLL B-cells *in vitro* [12]. EGCG is the main polyphenol present in green tea. EGCG and curcumin are both capable of inducing poly (ADP-ribose) polymerase (PARP) cleavage and inhibiting telomerase activity in CLL B-cells [12]. Curcumin does not activate caspase-3 whereas EGCG does activate caspase-3 while inducing PARP cleavage and apoptosis in B-CLL [12]. EGCG and curcumin in 10:1 ratio has been observed to be effective in causing apoptosis in CLL B-cells [12]. Also to be known is that high doses of curcumin and EGCG are required to overcome stromal protection in these patients [12]. A case of complete remission of CLL has been observed through the administration of EGCG at a dose of 1200 mg/day [39]. This patient was also given curcumin at the dose of 4 g daily [39].

In an *in vitro* study conducted by Everett *et al*. curcumin induced dose-dependent apoptosis in CLL B-cells. The study observed that 10 μM curcumin induced optimal apoptosis of CLL B-cells after 24–48 h of exposure by downregulating NF-kappa B signaling. The study also observed that nonmalignant mononuclear cells were less sensitive when compared to CLL B-cells. Curcumin showed a supra-additive effect when administered with rolipram and vincristine by augmenting apoptosis. Curcumin exhibited either no effect or supra-additive effect when administered with fludarabine and dexamethasone. It is to be known that even to achieve 1 μM serum levels of curcumin, large oral doses of curcumin are required. The active ingredient of black pepper-piperine is known to increase the bioavailability of curcumin by 2000% [40]. However, piperine is observed to be a potent inhibitor of drug metabolism and hence can cause drug toxicity [41,42].

Curcumin inhibits NIK/IKK complex and IKB kinase contributing to its antiproliferative and proapoptotic activity. Hayun *et al*. in their study observed that 10 μM curcumin induces apoptosis in CLL B-cells by caspase activation, Bcl-2 downregulation, and upregulation of Bax protein. The study suggested that curcumin effectively induces apoptosis in resting CLL B-cells [43].

4. Dosage of curcumin in cancer

Most commonly prescribed curcumin formulation in various cancers is Curcumin C3 complex with the dosage ranging from 3.6 to 8 g daily [17]. A single capsule of this formulation contains 450 mg curcumin [17]. Daily oral dose of 3.6 g curcumin appears to be convenient and suitable [44]. Literature documents that in order for cancer cells to die, they should be exposed to 5–50 μM curcumin concentration for several hours [45]. It would be useful to know that pure turmeric approximately contains curcumin in concentrations of 3.14% by weight [46]. It is estimated that curcuminoid content of turmeric approximately ranges from 2% to 6% by weight [47].

5. Curcumin toxicity

Curcumin in the dose of 0–3 mg/kg bodyweight is defined as acceptable daily intake by FAO/WHO expert committee [19]. It is observed to be safe in humans and rodents when administered up to 10 g/day [19]. Research indicates that humans can tolerate a high dose of curcumin as high as 12 g/day without toxic effects [16]. Curcumin, in humans, is well tolerated at a dose of 8 g/day [21]. In few patients, curcumin can cause diarrhea and nausea which can be easily managed [44]. Few patients are noted to exhibit increased levels of lactate dehydrogenase and serum alkaline phosphatase on the intake of 0.45–3.6 g/day curcumin for one to four months [44]. However, the authors suggested that curcumin could be used at a dose of 3.6 g/day for preventing or treating cancers present outside the gastrointestinal tract [44]. Lao *et al*. in their study, administered capsules containing standardized curcumin powder extract to healthy individuals at increasing doses from 500 mg to 12 g [48]. The study concluded that single, high oral doses of curcumin can be well tolerated. It is advised that curcumin should be used in caution with smokers because curcumin induced ROS can further damage the lungs [19]. A study by Tang *et al*. assessed urinary oxalate excretion in healthy subjects taking turmeric supplements (2.8 g) for four weeks. The turmeric supplements used in the study had an oxalate content of 1969 mg/100 ml. After four weeks, an oxalate load test with 63 mg turmeric was done. They observed higher urinary oxalate excretion in turmeric supplement intake group. The authors cautioned that increased turmeric intake may probably cause kidney stone formation in predisposed individuals [49]. Cao *et al*. observed that high curcumin concentrations- 2.5, 5 and 10–40 μg/mL, induced mitochondrial and DNA damage in human hepatoma G2 cells [50]. Curcumin is also observed to be a chelator of iron and to precipitate iron deficiency anemia *in vivo* in mice fed with a diet deficient in iron [45]. Also, curcumin is noted to inhibit several drug metabolizing enzymes such as glutathione-S-transferase, cytochrome P450, and UDP-glucuronosyltransferase. However, it should be noted that iron stimulates cancer initiation, tumor growth and metastasis [51]. Hence curcumin's iron chelating activity may be beneficial,

especially to cancer patients with excess iron. However, curcumin should be used with caution in iron deficient patients and also in patients taking drugs that are metabolized by enzymes inhibited by curcumin [45].

6. Limitations of curcumin

Limitations to the anticancer activity of curcumin are its low bioavailability, poor pharmacokinetics, poor absorption, slow cellular uptake, low water solubility, rapid metabolism and low bioavailability [14,19,52]. Curcumin is extensively metabolized in intestine and liver [45]. Bioavailability of curcumin can be increased by chemical modifications, decreasing hydrophobicity, by using nanoparticle and liposomal encapsulation formulations, increasing membrane permeability through the incorporation of it in micelles and phospholipids formulation [14,19]. Increased curcumin bioavailability can also be attempted through intravenous infusions [34]. The action of curcumin can be enhanced by combining it with other substances like quercetin, prednisone, etc [14]. Bioavailability of curcumin can also be enhanced by 12 fold by heat [52].

7. Conclusions and future perspectives

Currently, chronic lymphocytic leukemia is incurable by current treatment modalities. Also, hematopoietic stem cell transplantation and currently available anti-leukemic drugs have several side effects. These treatment modalities are also very costly and impose an additional economic burden in already emotionally down patients. Curcumin is readily available, safe, cost-effective natural product which is extensively researched with respect to cancer. Curcumin has anti-carcinogenic properties and multi-target actions and is beneficial in leukemia, especially in early stages of chronic lymphocytic leukemia. It prevents the progression of the disease, decreases CLL B-cell counts, and also when administered together with conventional anti-cancer drugs, has synergistic actions in addition to lowering their dose and side effects. Since curcumin has low bioavailability, more research should be conducted to develop new curcumin formulations by using nanotechnology, chemically modifying the hydrophobic component of curcumin or by combining or encapsulating it with other products/therapeutics. Curcumin, if it is used in anticancer therapy, it essentially needs to be taken for a long duration. However, currently, there are no long-term clinical trials assessing the safety of high dose curcumin in humans. Hence, to better realize the utility of curcumin as an anticancer drug in chronic lymphocytic leukemia or in any cancer, it is suggested that more long-term clinical studies on humans assessing the safety of high dose curcumin should be carried out. It is observed that curcumin-free turmeric also exhibits anticancer and anti-inflammatory activities [53]. Turmeric, in addition to curcumin, also contains turmerone, elemene, and furanodiene, etc, which may exhibit anti-leukemic activity. Turmeric oil present in turmeric is shown to enhance the bioavailability of curcumin [53]. Hence, it is also suggested that more long-term clinical trials assessing the anti-leukemic activity of turmeric should be carried out in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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