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Review On Effect Of Curcumin In The Treatment Of Cancer

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Abstract

Caner is a devastating disease condition and is the second most life taking disease globally. After decades of research in this field we are still looking for therapeutical agents with the most efficacies and least toxicities. Curcumin is one of the cancer therapeutic agents that is derived from the Curcuma longa(turmeric) plant, and still in vitro and in vivo research is going on to find its beneficial effects on various cancers. Due to its potency to affect multiple targets of different cellular pathways. It is considered a promising agent to tackle various cancers alone or in combination with the existing chemotherapies. This review covers basic properties, mechanism of action, potential targets of curcumin. This study aims to evaluate the antiproliferative property of curcuminoids in three human cancer cell lines. The study consists of a) Establishing inhibition-concentration 50% (IC_{50}), by Methyl thiazole tetrazolium (MTT) reduction method and b) Viable Cell Count by Trypan blue exclusion method.

INTRODUCTION

(I) Need for the Study:

Curcumin is one of the major derivative of *Curcuma longa* (turmeric). The other two derivatives are demethoxy curcumin and bis-demethoxy curcumin which together constitute upto ~20%. Numerous studies have indicated the anti-oncogenic property of curcumin. In addition to this, a few studies indicate the anti-cancer property of naturally occuring derivatives of curcumin. Many synthetic analogs of curcumin have been studied for development of cancer chemotherapy. However, the mechanism of action of these compounds remains to be enigmatic.

(II) Objectives of the Study

This study aims to evaluate the antiproliferative property of curcuminoids in three human cancer cell lines. The study consists of a) Establishing inhibition-concentration 50% (IC_{50}), by Methyl thiazole tetrazolium (MTT) reduction method and b) Viable Cell Count by Trypan blue exclusion method.

REVIEW OF RELATED LITERATURE

A. CELL BIOLOGY OF CANCER Cancer in all forms causes about 12% of deaths throughout the world. In the developed countries cancer is the second leading cause of death accounting for 21% (2.5 million) of all

mortality. Cancer has become one of the ten leading causes of death in India. According to an estimate there are nearly 1.5-2 million cancer cases at any given point of time. Data from population-based registries under National Cancer Registry Programme (Press Information Bureau, Government of India, 2001) indicate that the leading sites of cancer are oral cavity, lungs, esophagus and stomach amongst men and cervix, breast and oral cavity amongst women. Cancers of mouth and lungs in males, and cervix and breast in females account for over 50% of all cancer deaths in India.The three most common cancers in the U.S. in men are prostate, lung and colon cancer and in women being breast, lung, and colon cancer.

The mutations that produce oncogenes with dominant gain of function and tumor suppressor genes with recessive loss of function; both classes of cancer genes have been identified through their alteration in human and animal cancer cells and by their elicitation of cancer phenotypes in experimental models (Bishop and Weinberg, 1996). Many types of cancers are diagnosed in the human population with an age-dependent incidence implicating four to seven rate-limiting, stochastic events (Renan, 1993).
Pathological analyses of a number of organ sites reveal lesions that appear to represent the intermediate steps in a process through which cells evolve progressively from normalcy via a series of premalignant states into invasive cancers (Foulds, 1954). These observations have been rendered more concrete by a large body of work indicating that the genomes of tumor cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement (e.g., Kinzler and Vogelstein, 1996).

The vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These six capabilities are shared in common by most and perhaps all types of human tumors (Hanahan and Weinberg, 2000). Each of these physiologic changes—novel capabilities acquired during tumor development—represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues.

Acquired G1-S phase of cell cycle (GS) autonomy was the first of the six capabilities to be clearly defined by cancer researchers, in large part because of the prevalence of dominant oncogenes that have been found to modulate it. Three common molecular strategies for achieving autonomy are evident, involving alteration of extracellular growth signals, of transcellular transducers of those signals, or of intracellular circuits that translate those signals into action. While most soluble mitogenic growth-factors (GFs) are made by one cell type in order to stimulate proliferation of another—the process of heterotypic signaling—many cancer cells acquire the ability to synthesize GFs to which they are responsive, creating a positive feedback signaling loop often termed autocrine stimulation (Fedi et al., 1997). Clearly, the manufacture of a GF by a cancer cell obviates dependence on GFs from other cells within the tissue. The production of Platelet derived growth factor (PDGF) and Transforming growth factor-alpha (TGFα) by glioblastomas and sarcomas, respectively, are two illustrative examples (Fedi et al., 1997).

The cell surface receptors that transduce growth stimulatory signals into the cell interior are themselves targets of deregulation during tumor pathogenesis. GF receptors, often carrying tyrosine kinase activities in their cytoplasmic domains, are overexpressed in many cancers. Receptor overexpression may enable the cancer cell to become hyperresponsive to ambient levels of GF that normally would not trigger proliferation (Fedi et al., 1997). For example, the epidermal growth factor-receptor (EGF-R/*erbB*) is upregulated in stomach, brain, and breast tumors, while the HER2/*neu* receptor is overexpressed in stomach and mammary carcinomas (Slamon et al., 1987; Yarden and Ullrich, 1988). Additionally, gross overexpression of GF receptors can elicit ligand-independent signaling (DiFiore et al., 1987). Ligand-independent signaling can also be achieved through structural alteration of receptors; for example, truncated versions of the EGFR lacking much of its cytoplasmic domain fire constitutively (Fedi et al., 1997).

Cancer cells can also switch the types of extracellular matrix receptors (integrins) they express, favoring ones that transmit progrowth signals (Lukashev and Werb, 1998; Giancotti and Ruoslahti, 1999). These bifunctional, heterodimeric cell surface receptors physically link cells to extracellular superstructures known as the extracellular matrix (ECM). Successful binding to specific moieties of the ECM enables the integrin receptors to transduce signals into the cytoplasm that influence cell behavior, ranging from quiescence in normal tissue to motility, resistance to apoptosis, and entrance into the active cell cycle. Conversely, the failure

of integrins to forge these extracellular links can impair cell motility, induce apoptosis, or cause cell cycle arrest (Giancotti and Ruoslahti, 1999). Both ligand-activated GF receptors and progrowth integrins engaged to ECM components can activate the SOS-Ras-Raf-MAP kinase pathway (Aplin et al., 1998; Giancotti and Ruoslahti, 1999). The most complex mechanisms of acquired GS-autonomy derive from alterations in components of the downstream cytoplasmic circuitry that receives and processes the signals emitted by ligand-activated GF receptors and integrins. The SOS-Ras-Raf-MAPK cascade plays a central role here. In about 25% of human tumors, Ras proteins are present in structurally altered forms that enable them to release a flux of mitogenic signals into cells, without ongoing stimulation by their normal upstream regulators (Medema and Bos, 1993). Ras-oncogene mutation is characterized in human colon carcinoma.

Within normal tissue, cells are largely instructed to grow by their neighbors (paracrine signals) or via systemic (endocrine) signals. Cell-to-cell growth signaling is likely to operate in the vast majority of human tumors as well; virtually all are composed of several distinct cell types that appear to communicate via heterotypic signaling. In addition, multiple antiproliferative signals operate to maintain cellular quiescence and tissue homeostasis; these signals include both soluble growth inhibitors and immobilized inhibitors embedded in the extracellular matrix and on the surfaces of nearby cells. These growth-inhibitory signals, like their positively acting counterparts, are received by transmembrane cell surface receptors coupled to intracellular signaling circuits. Antigrowth signals can block proliferation by two distinct mechanisms. Cells may be forced out of the active proliferative cycle into the quiescent (G0) state from which they may reemerge on some future occasion when extracellular signals permit. Alternatively, cells may be induced to permanently relinquish their proliferative potential by being induced to enter into postmitotic states, usually associated with acquisition of specific differentiation-associated traits.

Incipient cancer cells must evade these antiproliferative signals if they are to prosper. Much of the circuitry that enables normal cells to respond to antigrowth signals is associated with the cell cycle clock, specifically the components governing the transit of the cell through the G1 phase of its growth cycle. Cells monitor their external environment during this period and, on the basis of sensed signals, decide whether to proliferate, to be quiescent, or to enter into a postmitotic state.

At the molecular level, many and perhaps all antiproliferative signals are funneled through the Retinoblastoma protein (pRb) and its two relatives, p107 and p130. When in a hypophosphorylated state, pRb blocks proliferation by sequestering and altering the function of E2F transcription factors that control the expression of banks of genes essential for progression from G1 into S phase of the cell cycle (Weinberg, 1995). Disruption of the pRb pathway liberates E2Fs and thus allows cell proliferation, rendering cells insensitive to antigrowth factors that normally operate along this pathway to block advance through the G1 phase of the cell cycle. The effects of the soluble signaling molecule Transforming growth factor-beta (TGFβ) are the best documented, but we envision other antigrowth factors which are found to signal through this pathway as well. TGFβ acts in a number of ways, most still elusive, to prevent the phosphorylation that inactivates pRb; in this fashion, TGFβ blocks advance through G1. In some cell types, TGFβ suppresses expression of the c-myc gene, which regulates the G1 cell cycle machinery in still unknown ways (Moses et al., 1990). More directly, TGFβ causes synthesis of the $p15^{INK4B}$ and p21 proteins, which block the cyclin:Cyclin dependent kinase (CDK) complexes responsible for pRb phosphorylation (Hannon and Beach, 1994; Datto et al., 1997). The pRb signaling circuit, as governed by TGFβ and other extrinsic factors, can be disrupted in a variety of ways in different types of human tumors (Fynan and Reiss, 1993). Some lose TGFβ responsiveness through downregulation of their TGFβ receptors, while others display mutant, dysfunctional receptors (Fynan and Reiss, 1993; Markowitz et al., 1995). The cytoplasmic Smad-4 protein, which transduces the signals from ligand-activated TGFβ receptors to downstream targets, may be eliminated through mutation of its encoding gene Schutte et al., 1996). The locus encoding $p15^{INK4B}$ may be deleted (Chin et al., 1998). Alternatively, the immediate downstream target of its actions, CDK4, may become unresponsive to the inhibitory actions of $p15^{INK4B}$ because of mutations that create amino acid substitutions in its INK4A/B-interacting domain; the resulting cyclin D: CDK4 complexes are then given a free hand to inactivate pRb by hyperphosphorylation (Zuo et al., 1996). Finally, functional pRb, the end target of this pathway, may be lost through mutation of its gene. Alternatively, in certain deoxyribose nucleic acid (DNA) virus-induced tumors, notably cervical carcinomas, pRb function is eliminated through sequestration by viral oncoproteins, such as the E7 oncoprotein of human papillomavirus (Dyson et al., 1989).

In addition, cancer cells can also turn off expression of integrins and other cell adhesion molecules that send antigrowth signals, favoring instead those that convey progrowth signals; these adherence-based antigrowth signals likely impinge on the pRb circuit as well. The bottom line is that the antigrowth circuit converging onto Rb and the cell division cycle is one way or another, disrupted in a majority of human cancers, defining the concept and a purpose of tumor suppressor loss in cancer. Cell proliferation depends on more than an avoidance of cytostatic antigrowth signals. Our tissues also constrain cell multiplication by instructing cells to enter irreversibly into postmitotic, differentiated states, using diverse mechanisms that are incompletely understood; is apparent that tumor cells use various strategies to avoid this terminal differentiation. One strategy for avoiding differentiation directly involves the c-myc oncogene, which encodes a transcription factor. During normal development, the growth stimulating action of myc in association with another factor, max, can be supplanted by alternative complexes of max with group of mad-transcription factors, the mad–max complexes elicit differentiation-inducing signals (Foley and Eisenman, 1999). However, overexpression of the c-myc oncoprotein, as is seen in many tumors, can reverse this process, shifting the balance back to favor Myc–Max complexes, thereby impairing differentiation and promoting growth. During human colon carcinogenesis, inactivation of the (APC)/β-catenin pathway serves to block the egress of enterocytes in the colonic crypts into differentiated, postmitotic state (Kinzler and Vogelstein, 1996).

Evasion of Apoptosis is a very important molecular hallmark of that has been observed during oncogenesis. The ability of tumor cell populations to expand in number is determined not only by the rate of cell proliferation but also by the rate of cell attrition. The apoptotic program is present in latent form in virtually all cell types throughout the body. Once triggered by a variety of physiologic signals, this program unfolds in a precisely choreographed series of steps. The cell membranes are disrupted, the cytoplasmic and nuclear skeletons are broken down, the cytosol is extruded, the chromosomes are degraded, and the nucleus is fragmented, all in a span of 30–120min. In the end, the shriveled cell corpse is engulfed by nearby cells in a tissue and disappears, typically within 24h (Wyllie et al., 1980).

The apoptotic machinery can be broadly divided into two classes of components—sensors and effectors. The sensors are responsible for monitoring the extracellular and intracellular environment for conditions of normality or abnormality that influence whether a cell should live or die. These signals regulate the second class of components, which function as effectors of apoptotic death. The sentinels include cell surface receptors that bind survival or death factors. Examples of these ligand receptor pairs include survival signals conveyed by Insulin like growth factor-1 (IGF)/IGF-2 through their receptor, IGF-1R, and by interleukin (IL)-3 and its cognate receptor, IL-3R (Lotem and Sachs, 1996; Butt et al., 1999). Death signals are conveyed by the FAS ligand binding the FAS receptor and by TNFα binding TNF-R1 (Ashkenazi and Dixit, 1999). Intracellular sensors monitor the cell's well-being and activate the death pathway in response to detecting abnormalities, including DNA damage, signaling imbalance provoked by oncogene action, survival factor insufficiency, or hypoxia (Evan and Littlewood, 1998). Further, the life of most cells is in part maintained by cell–matrix and cell–cell adherence-based survival signals whose abrogation elicits apoptosis (Ishizaki et al., 1995; Giancotti and Ruoslahti, 1999). Both soluble and immobilized apoptotic regulatory signals likely reflect the needs of tissues to maintain their constituent cells in appropriate architectural configurations.

Many of the signals that elicit apoptosis converge on the mitochondria, which respond to proapoptotic signals by releasing cytochrome C, a potent catalyst of apoptosis (Green and Reed, 1998). Members of the Bcl-2 family of proteins, whose members have either proapoptotic (Bax, Bak, Bid, Bim) or antiapoptotic (Bcl-2, Bcl-XL, Bcl-W) function, act in part by governing mitochondrial death signaling through cytochrome C release. The p53 tumor suppressor protein can elicit apoptosis by upregulating expression of proapoptotic Bax in response to sensing DNA damage; Bax in turn stimulates mitochondria to release cytochrome C. The ultimate effectors of apoptosis include an array of intracellular proteases termed caspases (Thornberry and Lazebnik, 1998). Two "gatekeeper" caspases, -8 and -9, are activated by death receptors such as FAS or by the cytochrome C released from mitochondria, respectively. These proximal caspases trigger the activation of a dozen or more effector caspases that execute the death program, through selective destruction of subcellular structures and organelles, and of the genome.

The possibility that apoptosis serves as a barrier to cancer was first raised in 1972, when Kerr, Wyllie, and Currie described massive apoptosis in the cells populating rapidly growing, hormone-dependent tumors following hormone withdrawal (Kerr et al., 1972). The discovery of the bcl-*2* oncogene by its upregulation via chromosomal translocation in follicular lymphoma (reviewed in Korsmeyer, 1992) and its recognition as

having antiapoptotic activity (Vaux et al., 1988) opened up the investigation of apoptosis in cancer at the molecular level.

The most commonly occurring loss of a proapoptotic regulator mutation involves the p53 tumor suppressor gene. The resulting functional inactivation of its product, which is seen in greater than 50% of human cancers and causes removal of a key component of the DNA damage sensor that can induce the apoptotic effectors cascade (Harris, 1996). Signals evoked by other abnormalities, including hypoxia and oncogene hyperexpression, are also funneled in part via p53 to the apoptotic machinery; these too are impaired at eliciting antiapoptosis when p53 function is lost (Levine, 1997). Additionally, the phospho inositol-3 kinase (PI3K)–AKT/protein kinase-B (PKB) pathway, which transmits antiapoptotic survival signals, is likely involved in mitigating apoptosis in a substantial fraction of human tumors. This survival signaling circuit can be activated by extracellular factors such as IGF-1/2 or IL-3 (Evan and Littlewood, 1998), by intracellular signals emanating from Ras (Downward, 1998), or by loss of the pTEN tumor suppressor, a phospholipid phosphatase that normally attenuates the AKT survival signal (Cantley and Neel, 1999). Recently, a mechanism for abrogating the FAS death signal has been revealed in a high fraction of lung and colon carcinoma cell lines: a nonsignaling decoy receptor for FAS ligand is upregulated, titrating the death-inducing signal away from the FAS death receptor (Pitti et al., 1998).

Three acquired capabilities—growth signal autonomy, insensitivity to antigrowth signals, and resistance to apoptosis—all lead to an uncoupling of a cell's growth program from signals in its environment. In principle, the resulting deregulated proliferation program should suffice to enable the generation of the vast cell populations that constitute macroscopic tumors.

The early work of Hayflick demonstrated that cells in culture have a finite replicative potential (reviewed in Hayflick, 1997). Once such cell populations have progressed through a certain number of doublings, they stop growing—a process termed senescence. For example: senescence of cultured human fibroblasts can be circumvented by disabling their pRb and p53 tumor suppressor proteins, enabling these cells to continue multiplying for additional generations until they enter into a second state termed crisis. The crisis state is characterized by massive cell death, karyotypic disarray associated with end-to-end fusion of chromosomes, and the occasional emergence of a variant (1 in 10^7) cell that has acquired the ability to multiply without limit, the trait termed immortalization (Wright et al., 1989). Provocatively, most types of tumor cells that are propagated in culture appear to be immortalized, suggesting limitless replicative potential is a phenotype that was acquired in vivo during tumor progression and was essential for the development of their malignant growth state (Hayflick, 1997). This result suggests that at some point during the course of multistep tumor progression, evolving premalignant cell populations exhaust their endowment of allowed doublings and can only complete their tumorigenic agenda by breaching the mortality barrier and acquiring unlimited replicative potential. the ends of chromosomes, telomeres, which are composed of several thousand repeats of a short 6 bp sequence element. Replicative generations are counted by the 50–100 bp loss of telomeric DNA from the ends of every chromosome during each cell cycle. This progressive shortening has been attributed to the inability of DNA polymerases to completely replicate the 39 ends of chromosomal DNA during each S phase. The progressive erosion of telomeres through successive cycles of replication eventually causes them to lose their ability to protect the ends of chromosomal DNA. The unprotected chromosomal ends participate in end-to-end chromosomal fusions, yielding the karyotypic disarray associated with crisis and resulting, almost inevitably, in the death of the affected cell (Counter et al., 1992). Telomere maintenance is evident in virtually all types of malignant cells (Shay and Bacchetti, 1997); 85%–90% of them succeed in doing so by upregulating expression of the telomerase enzyme, which adds hexanucleotide repeats onto the ends of telomeric DNA (Bryan and Cech, 1999), while the remainder have invented a way of activating a mechanism, termed ALT, which appears to maintain telomeres through recombination-based interchromosomal exchanges of sequence information (Bryan et al., 1995). By one or the other mechanism, telomeres are maintained at a length above a critical threshold, and this in turn permits unlimited multiplication of descendant cells. Both mechanisms seem to be strongly suppressed in most normal human cells in order to deny them unlimited replicative potential.

The role of telomerase in immortalizing cells can be demonstrated directly by ectopically expressing the enzyme in cells, where it can convey unlimited replicative potential onto a variety of normal early passage, cells in vitro (Bodnar et al., 1998; Vaziri and Benchimol, 1998). Further, late passage cells poised to enter crisis continue to proliferate without giving any evidence of crisis when supplied with this enzyme (Counter

et al., 1998; Halvorsen et al., 1999; Zhu et al., 1999). More recently, the senescent state has been observed to be inducible in certain cultured cells in response to high-level expression of genes such as the activated *ras* oncogene (Serrano et al., 1997). Thus activation of telomerase is key factor that looked upon in development of newer drugs.

In order to progress to a larger size, incipient neoplasias must develop angiogenic ability (Bouck et al., 1996; Hanahan and Folkman, 1996; Folkman, 1997). Among the many discovered angiogenic factors the VEGF, acidic- and basic- Fibroblast growth factor (FGF) have been best studied. Each binds to transmembrane tyrosine kinase receptors displayed by endothelial cells (Fedi et al., 1997; Veikkola and Alitalo, 1999). A prototypical angiogenesis inhibitor is thrombospondin-1, which binds to CD36, a transmembrane receptor on endothelial cells coupled to intracellular Src-like tyrosine kinases (Bull et al., 1994). There are currently more than dozen angiogenic inducer factors known and a similar number of endogenous inhibitor proteins. During oncogenesis, angiogenic factors are upregulated. In others, expression of endogenous inhibitors such as thrombospondin-1, β-interferon is downregulated. Moreover, both transitions may occur, and indeed be linked, in some tumors Singh et al., 1995; Volpert et al., 1997). The mechanisms underlying shifts in the balances between angiogenic regulators remain incompletely understood. Integrin signaling also contributes to this regulatory balance. Quiescent vessels express one class of integrins, whereas sprouting capillaries express another. Interference with signaling from the latter class of integrins can inhibit angiogenesis (Varner and Cheresh, 1996; Giancotti and Ruoslahti, 1999), underscoring the important contribution of cell adhesion to the angiogenic program (Hynes and Wagner, 1996). Extracellular proteases are physically and functionally connected with proangiogenic integrins, and both help dictate the invasive capability of angiogenic endothelial cells (Stetler-Stevenson, 1999).

The ability to induce and sustain angiogenesis seems to be acquired in a discrete step (or steps) during tumor development, via an “angiogenic switch” from vascular quiescence. Tumors appear to activate the angiogenic switch changing the balance of angiogenesis inducers and countervailing inhibitors (Hanahan and Folkman, 1996). The mechanisms underlying shifts in the balances between angiogenic regulators remain incompletely understood. In one well-documented example, the inhibitor thrombospondin-1 has been found to positively regulate by the p53 tumor suppressor protein in some cell types. Consequently, loss of p53 function, which occurs in most human tumors, can cause thrombospondin-1 levels to fall, liberating endothelial cells from inhibitory effects (Dameron et al., 1994). The Vascular enodothelial growth factor (VEGF) gene is also under complex transcriptional control.

Another dimension of regulation is emerging in the form of proteases, which can control the bioavailability of angiogenic activators and inhibitors. Thus, a variety of proteases can release basic-FGF stored in the ECM (Whitelock et al., 1996), whereas plasmin, a proangiogenic component of the clotting system, can cleave itself into an angiogenesis inhibitor form called angiostatin (Gately et al., 1997). The coordinated expressions of pro- and anti-angiogenic signaling molecules, and their modulation by proteolysis, appear to reflect the complex homeostatic regulation of normal tissue angiogenesis and of vascular integrity. As is already apparent, tumor angiogenesis offers uniquely attractive therapeutic target, indeed one that is shared in common by most and perhaps all types of human tumors.

During the development of most types of human cancer, primary tumor masses spawn pioneer cells that move out, invade adjacent tissues, and thence travel to distant sites where they may succeed in forming new colonies. These distant settlements of tumor cells—metastases—are the cause of 90% of human cancer deaths (Sporn, 1996). The capability for invasion and metastasis enables cancer cells to escape the primary tumor mass and colonize new terrain in the body where, at least initially, nutrients and space are not limiting. The newly formed metastases arise as amalgams of cancer cells and normal supporting cells conscripted from the host tissue. Like the formation of the primary tumor mass, successful invasion and metastasis depend upon all of the other five acquired hallmark capabilities. Invasion and metastasis are exceedingly complex processes, and their genetic and biochemical determinants remain incompletely understood. At the mechanistic level, they are closely allied processes, which justify their association with one another as one general capability of cancer cells. Both utilize similar operational strategies, involving changes in the physical coupling of cells to their microenvironment and activation of extracellular proteases. Several classes of proteins involved in the tethering of cells to their surroundings in a tissue are altered in cells possessing invasive or metastatic capabilities. The affected proteins include Cell adhesion molecule (CAM) s—notably members of the immunoglobulin and calcium-dependent cadherin families (integral membrane glycoprotein), both of which mediate cell-to-cell interactions—and

integrins, which link cells to extracellular matrix substrates. E-cadherin cytoplasmic domains bind complexes containing α- and β-catenins, which are structurally linked to the cytoskeleton (actin cables and intermediate filaments). β-catenin that is not sequestered in E-cadherin complexes is rapidly phosphorylated by Glycogen synthase kinase (GSK)-3 β in a complex with the adenomatous polyposis coli (APC) gene product (map to chromosome 5, mutated in familial polyposis) and is degraded by the ubiquitin/proteasome pathway. Degradation of β-catenin can be blocked by several mechanisms; including mutations that inactivate APC and mutations in serine phosphorylation sites within β-catenin that target it for degradation. Such mutations result in increased free β-catenin, which translocates into nucleus and binds to members of the T-cell factor family of transcription factors (Christofori and Semb, 1999 influencing the expression of genes such as c-myc and cyclin-D1 that promote progression through G1. Excess free β-catenin has been implicated in hereditary and sporadic forms of colon cancers (Fogar et al., 1997) and melanoma. Decreased expression E-cadherin has been noted in breast, colon, prostate, gastric and other cancers and is a marker of poor prognosis.

Invading and metastasizing cancer cells experience changing tissue microenvironments during their journeys, which can present novel matrix components. Epithelial cell growth and survival require attachment of cells to components of the ECM that compose basement membranes, including collagen, fibronectin, vitronectin and laminin. The intregin family of transmembrane receptors is composed of α- and β- that adhere to the ECM and convey information to cytoplasmic membrane-associated structure of focal adhesions. These novel permutations result in different integrin subtypes (of which there are greater than 22) having distinct substrate preferences. The Integrin-ECM complexes is mediated by the Rho and Tac GTPases, are sites of attachment of actin cables but are also active in cell signaling through their association with focal adhesion kinase are Src tyrosine kinases. Integrin-ECM interactions lead to activation of the Ras/MAP kinase, PI3K, and PLC-γ pathways. Detachment of epithelial and endothelial cells from ECM induces their death by a form of programmed called aniokis (Greek, "homeless"). This molecular safeguard prevents abnormal spread of cells. Invasive cancers often avoid anoikis by activating Ras or Src, which allow anchorage-independent growth of cells by activation of Akt kinase.

The second general parameter of the invasive and metastatic capability involves extracellular proteases (Coussens and Werb, 1996; Chambers and Matrisian, 1997). Protease genes are upregulated, protease inhibitor genes are downregulated, and inactive zymogen forms of proteases are converted into active enzymes. Matrix-degrading proteases are characteristically associated with the cell surface, by synthesis with a transmembrane domain, binding to specific protease receptors, or association with integrins (Werb, 1997; Stetler-Stevenson, 1999). In many types of carcinomas, matrix-degrading proteases are produced not by the epithelial cancer cells but rather by conscripted stromal and inflammatory cells (Werb, 1997). For example, certain cancer cells induce urokinse plasminogen activator (uPA) expression in co-cultured stromal cells, which then binds to the uPA-receptor (uPAR) expressed on the cancer cells (Johnsen et al., 1998). The activation of extracellular proteases and the altered binding specificities of cadherins, CAMs, and integrins are clearly central to the acquisition of invasiveness and metastatic ability. But the regulatory circuits and molecular mechanisms that govern these shifts remain elusive and, at present, seem to differ from one tissue environment to another. The acquired capability for invasion and metastasis represents the last great frontier for exploratory cancer research.

B. Curcumin and Cancer Chemoprevention

An increasing attention is being paid to the possibility of applying cancer chemopreventive agents for individuals at high risk of neoplastic development. For this purpose, the natural compounds have practical advantages with regard to availability, suitability for oral application, regulatory approval and mechanisms of action. Candidate substances such as phytochemicals present in foods and their derivatives have been identified by a combination of epidemiological and experimental studies. Plant constituents include vitamin derivatives, phenolic and flavonoid agents, organic sulfur compounds, isothiocyanates, curcumins, fatty acids and d-limonene. Examples of compounds from animals are unsaturated fatty acids and lactoferrin. Recent studies have indicated that mechanisms underlying chemopreventive potential may be combination of anti-oxidant, antiflammatory, proapoptotic, antiangiogenic, immune-enhancing and anti-hormone effects, with modification of drug-metabolizing enzymes, influence on the cell cycle progression and cell differentiation playing roles in the initiation and secondary modification stages of neoplastic development. Accordingly, natural agents are advantageous for application to humans because of their combined mild mechanism.

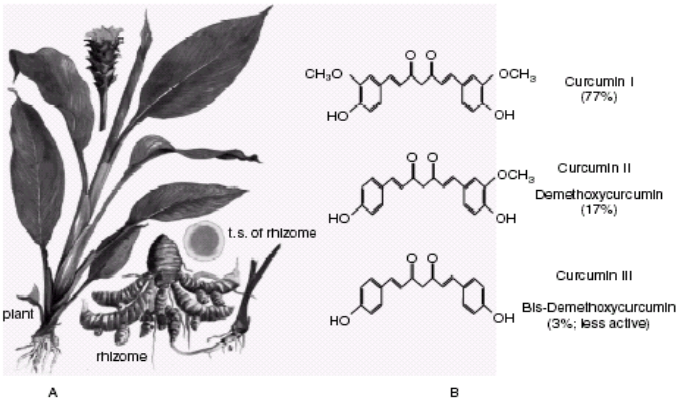

FIG.1.1: THE PLANT CURCUMA LONGA (PANEL A) FROM WHICH CURCUMIN IS DERIVED AND IT'S STRUCTURE (PANEL B)

Curcuma longa or turmeric is a tropical plant native to southern and southeastern tropical Asia. A perennial herb belonging to the ginger family, turmeric measures up to 1 m high with a short stem and tufted leaves (Fig. 1.1A). The parts used are the rhizomes. Perhaps the most active component in turmeric is curcumin, which may make up 2 to 5% of the total spice in turmeric (Fig. 1.1B). Curcumin is a diferuloylmethane present in extracts of the plant. Curcuminoids are responsible for the yellow color of turmeric and curry powder. They are isolated from turmeric by ethanol extraction. The pure orange-yellow, crystalline powder is insoluble in water. The structure of curcumin ($C_{21}H_{20}O_6$) was first described in 1815 by Vogel and Pellatier and in 1910 was shown to be diferuloylmethane (Lampe et al., 1910). Chemical synthesis in 1913 confirmed its identity (Lampe et al., 1913). Turmeric is widely consumed in the countries of its origin for a variety of uses, including as a dietary spice, a dietary pigment, and an Indian folk medicine for the treatment of various illnesses. It is used in the textile and pharmaceutical industries (Srimal and Dhawan, 1973) and in Hindu religious ceremonies in one form or another. Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis (Jain and DeFilipps, 1991). The old Hindu texts have described it as an aromatic stimulant and carminative (Nadkarni, 1954). Powder of turmeric mixed with slaked lime is a household remedy for the treatment of sprains and swelling caused by injury, applied locally over the affected area. In some parts of India, the powder is taken orally for the treatment of sore throat. This nonnutritive phytochemical is pharmacologically safe, considering that it has been consumed as a dietary spice, at doses up to 100 mg/day, for centuries (Amman and Wahl, 1991). Recent phase I clinical trials indicate that people can tolerate a dose as high as 8 g/day (Cheng, 2001). In the U.S., curcumin is used as a coloring agent in cheese, spices, mustard, cereals, pickles, potato flakes, soups, ice creams, and yogurts.

Curcumin is not water-soluble, but it is soluble in ethanol or in dimethyl sulfoxide. The degradation kinetics of curcumin under various pH conditions and the stability of curcumin in physiological matrices have been established (Wang, 1997). Curcumin decomposes upto 90% within 30 min when it was incubated in 0.1M phosphate buffer and serum-free medium (pH 7.2 at 37°C). A series of pH conditions ranging from 3 to 10 were tested and the results showed that decomposition was pH-dependent and occurred faster at neutral-basic conditions. It is more stable in cell culture medium containing 10% fetal calf serum and in human blood. Less than 20% of curcumin decomposed within 1 h, and after incubation for 8 h, about 50% of curcumin still remained. Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal was predicted to be the major degradation product, and vanillin, ferulic acid, and feruloyl methane were identified as minor degradation products. The amount of vanillin increased with incubation time. Numerous studies have indicated that curcumin has antioxidant and anti-inflammatory properties. A medline search revealed over 1000 publications describing various activities of this polyphenol.

Numerous reports suggest that curcumin has chemopreventive and chemotherapeutic effects (Fig. 1.2). Curcumin blocks tumor initiation induced by benzo[a]pyrene and 7,12dimethylbenz[a]anthracene (Huang et al., 1992) and it suppresses phorbol ester-induced tumor promotion (Huang et al., 1998, Conney et al., 1991). In vivo, curcumin was found to suppress carcinogenesis of the skin (Conney et al., 1991; Lu et al., 1994; Limtrakul et al., 1997 and Huang et al., 1997), the fore stomach (Huang et al., 1994 and Piper et al., 1998), the colon (Rao et al., 1995; Kim et al., 1998 and Kawamori et al., 1999), and the liver (Chuang et al., 2000) in mice. Curcumin also suppresses mammary carcinogenesis (Singletary et al., 1996; Chan et al., 1998 and Inano

et al., 1999). HER2/neu and EGFR activity is shown to be downregulated by curcumin and thus suppresses the growth of breast cancer cells. Curcumin has been shown to inhibit the proliferation of a wide variety of tumor cells including B cell and T cell leukemia (Kuo et al., 1996; Han et al., 1999; Piwocka et al., 1999 and Abe et al., 1999), colon carcinoma (Chen et al., 1999) and epidermoid carcinoma cells (Korutla and Kumar, 1994). It has been shown to suppress the proliferation of various carcinoma cell lines in culture (Mehta et al., 2001; Ramachandran and You, 1999).

Nuclear factor-kappa B (NF-kB) is a nuclear transcription factor required for the expression of genes involved in cell proliferation, cell invasion, metastasis, angiogenesis and resistance to chemotherapy (Baldwin, 2001). This factor is activated in response to inflammatory stimuli, carcinogens, tumor promoters, ad hypoxia, which frequently are encountered in tumor tissues (Wang et al., 1996; Lee et al., 1995; and Giri and Aggarwal, 1998), and it has been implicated in chemoresistance (Wang et al., 1996). Curcumin may also operate through suppression of NF-κB activation.

Overexpresssion of Cyclo oxygenase-2 (COX-2) has been shown to be associated with a wide variety of cancers, including colon (Fournier and Gordon, 2000) lung (Hida et al., 1998), and breast cancers (Harris et al., 2000). The role of COX-2 in suppression of apoptosis and tumor cell proliferation has been demonstrated (Williams et al., 1999). Furthermore, Celebrex, a specific inhibitor of COX-2, has been shown to suppress mammary carcinogenesis in animals (Reddy et al., 2000). Several groups have shown that curcumin down-regulates the expression of COX-2 protein in different tumor cells (Chen et al., 1999 and Plummer et al., 1999), most likely through the down-regulation of activation (Plummer et al., 1999), which is needed for COX-2 expression.

Cyclin D1, a component subunit of cyclin-dependent kinase Cdk4 and Cdk6, is a rate-limiting factor in progression of cells through the first gap (G1) phase of the cell cycle (Pan et al., 2000). Many studies have shown that Cyclin D1 is overexpressed in many cancers including breast, esophagus, head and neck, and prostate (Bartkova et al., 1994; Adelaide et al., 1995; Caputi et al., 1999; Nishida et al., 1994; Gumbiner et al., 1999 and Drobnjak et al., 2000). It is possible that the antiproliferative effects of curcumin are due to inhibition of cyclin D1 expression. It has benn shown that curcumin can indeed down-regulate cyclin D1 expression (Bharti et al., 2003; Mukhopadhyay et al., 2001), and this down-regulation occurred at the transcriptional and posttranscriptional level.

Numerous reports suggest that IL-6 promotes survival and proliferation of various tumors, including multiple myeloma cells, through the phosphorylation of a cell signaling protein, signal transducers and activators of transcription-3 (STAT3). Thus agents that suppress STAT3 phosphorylation have potential for the treatment of multiple myeloma. Bharti et al. (2003) demonstrated that curcumin inhibited IL-6-induced STAT3 phosphorylation and consequent STAT3 nuclear translocation.

Curcumin inhibits tumor growth and metastasis in animals. Menon et al. (1995) reported Curcumin-induced inhibition of B16F10 melanoma lung metastasis in mice. Kuttan et al. (1985) examined the anticancer potential of Curcumin in vitro using tissue culture methods and in vivo in mice using Dalton's lymphoma cells grown as ascites. Curcumin decreases the proliferative potential and increases apoptotic potential of both androgen-dependent and androgen-independent prostate cancer cells in vitro, largely by modulating the apoptosis suppressor proteins and by interfering with the growth factor receptor signaling pathways as exemplified by EGF receptor. To extend these observations, studies have been done to demonstrate the anti-cancer potential of Curcumin in a nude mouse prostate cancer model (Dorai et al., 2001).

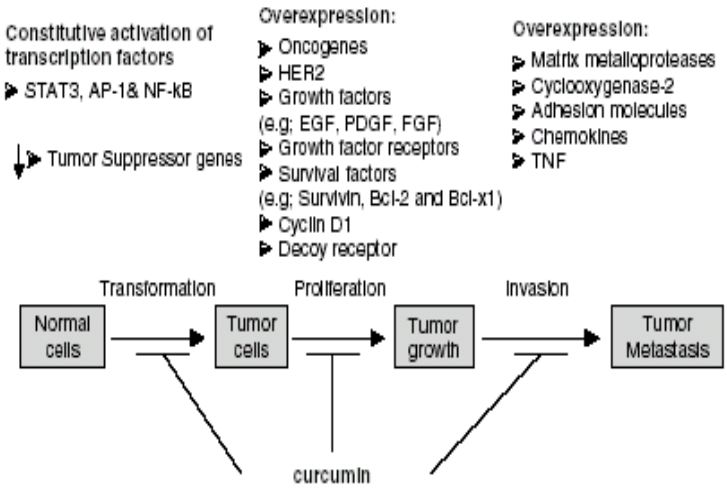

FIG.1. 2: VARIOUS STEPS INVOLVED IN TUMORIGENSIS AND METASTASIS AND THEIR SUPPRESSION BY CURCUMIN

The expression of various cell surface adhesion molecules such as intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1 on endothelial cells is absolutely critical for tumor metastasis (Ohene-Abuakwa and Pignatelli, 2000). The expression of these molecules is in part regulated by NF-kB (Iademarco et al., 1995).

For most solid tumors, including breast cancer, angiogenesis (blood vessel formation) is essential for tumor growth and metastasis (Folkman, 2001). The precise mechanism that leads to angiogenesis is not fully understood, but growth factors that cause proliferation of endothelial cells have been shown to play a critical role in this process. Curcumin has been shown to suppress the proliferation of human vascular endothelial cells in vitro (Singh et al., 1996) and abrogate the FGF-2 induced angiogenic response in vivo (Mohan et al., 2000), thus suggesting that curcumin is also an antiangiogenic factor. Indeed curcumin has been shown to suppress angiogenesis in vivo (Arbiser et al., 1998). To elucidate possible mechanisms of antiangiogenic activity by curcumin, Park et al. (2002), performed cDNA microarray analysis and found that curcumin modulated cell-cycle-related gene expression. Specifically, curcumin induced G0/G1- and G2/M-phase cell-cycle arrest; up-regulated CDKIs, $p21^{WAF1/CIP1}$, $p27^{KIP1}$, and p53; and slightly down-regulated cyclin B1 and cdc2 in ECV304 cells. The up-regulation of CDKIs by curcumin played a critical role in the regulation of cell-cycle distribution in these cells, which may underlie the antiangiogenic activity of curcumin.

The matrix metallo-proteinases (MMPs) make up a family of proteases that play a critical role in tumor metastasis (Kumar et al., 1999). It is shown that NF-kB activation leads to up-regulation of MMP-9, and curcumin leads to suppression of its expression (Lin et al., 1999). Curcumin has also been demonstrated to down-regulate inducible Nitric oxide synthase (iNOS) expression, also regulated by NF-kB and involved in tumor metastasis (Pan et al., 1999). These observations suggest that curcumin must have anti-metastatic activity. Indeed, there is a report suggesting that curcumin inhibits tumor metastasis (Menon et al., 1999).

METHODOLOGY

Plant material

The fresh rhizome of Curcuma longa was precured from the local market. The material was cut into small pieces and shade dried. The dried material was pulverized to a coarse powder.

Preparation of Extract:

The coarsely powdered (5Kg) rhizome was soaked in 20L of dichloromethane and kept for two days. The extraction was repeated twice with the residual material. The dichloromethane extracts were combined and concentrated to dryness in a rotatory evaporator at 40-45ºC under vacuum to yield 0.75 Kg of curcuminoids oleoresin.

Isolation of Curcuminoids by Column Chromatography:
About 100g of dichloromethane extract was suspended in chloroform and made into slurry with 500 g of silica gel (60-120 mesh). The column was packed with silica gel in n-hexane and chloroform (50:50) solvent mixture. The slurry poured on to the column and run with n-hexane-chloroform (50:50), n-hexane-chloroform (10:90), chloroform, chloroform-methanol (99.25:0.75) and chloroform-methanol (98:2). About 280 fractions of 10 ml each were collected and subjected to thin layer chromatography (TLC) using chloroform-methanol (95:5) mobile phase over precoated TLC plates (e-Merck). Based on TLC, fraction from 83 to 157, 166 to 196 and 198 to 230 were combined and concentrated to dryness to yield Curcumin (I) (16g), Demethoxycurcumin (II) (12g) and Bi-Demethoxycurcumin (III) (10g) respectively.

Preparation of sterile extract
A known amount of compound (curcuminoids) was weighed and dissolved in Dimethyl sulphoxide (DMSO) to make a concentration of 50mg/ml. This was then diluted with Phosphate buffer saline (PBS) (Sigma, USA) to obtain 10mg/ml and filtered through 0.2µm sterile filters (Sartoius, Germany). The concentration of the filtrate was obtained by High performance liquid chromography (HPLC) by comparing with the known standard concentration.

Cell lines
Human cancer cell lines namely MCF-7 (breast cell carcinoma), HL-60 (Leukemia) and COLO 320 DM (Colon carcinoma) were procured from National Center for Cell Sciences (Pune, India).

Preparation of Culture Media
The following media were used: (a) MCF-7 was grown in DMEM (Sigma, USA). (b) HL-60 and COLO 320 DM were grown in RPMI 1640 (Hi media, India). The DMEM was reconstituted with 3.7g/L sodium bicarbonate (sigma, USA), and 100µg/ml streptomycin and 100U/ml penicillin. The RPMI 1640 was reconstituted with 2.0g/L sodium bicarbonate (Sigma, USA), 100µg/ml streptomycin and 100U/ml penicillin. The volume was made upto 1L before which the pH was adjusted to 7.2. The media is filter sterilized using 0.2 µm filters under vacuum. Media is stored at 4ºC till use. Prior to addition of media to the culture, respective media was supplemented with 10% foetal calf serum (FCS) (Sigma, USA).

Cell culture
Cells were supplemented with respective media. Cultures were maintained at 37ºC, in 5% CO_2/95% air Incubator/humidified atmosphere (NUAIRE, USA). The spent media of MCF-7 and COLO 320 DM were discarded and fresh growth medium was supplemented for every 3 days once with a split ratio of 1:3. The growth medium was supplemented to HL-60 every two days once with a split ratio of 1:4.
For long-term maintenance of these cell lines, periodic cryo preservation was performed. Briefly, a healthy confluent flask (upto70-80%) was selected. Cryo-media consisted of 20% FCS (Sigma, USA), 10% DMSO (Sigma, USA) in suitable media (as mentioned above). The cells were trypsinised (MCF-7) or scraped (COLO 320 DM) or spent media (HL-60) was taken into a sterile tube and then centrifuged at 1000rpm for 10 minutes at 4ºC. To the cell pellet thus obtained a suitable volume of cryo-media was added and pellet was gently triturated in it. Aliquots were prepared in cryovials and kept in Mr. frosty container (filled with Isopropyl alcohol) in -80ºC deep freezer for >4 h. Finally these cryo vials were plunged into Liquid Nitrogen container. After a known period (eg. a week), cell line(s) was revived to test the success of cryo preservation. Briefly, the cryo-vial(s) removed from the liquid-nitrogen container was immediately placed into water bath of 37ºC, which will immediately thaw of contents. Later the contents were then emptied into culture flask and supplemented by respective media (20% FCS). The cells were visualized under inverted microscope to check the presence viable cells.

Preparation of Trypsin/EDTA solution
Trypsin (0.2%, sigma, USA), EDTA (0.05%, sigma, USA) was dissolved in PBS (Sigma, USA). The pH of solution was adjusted 7.4. This solution was then filter-sterilized using 0.2µm filter and aliquoted to small volumes and stored at 4ºC till use.

Preparation of Single cell suspension
MCF-7 forms a monolayer, COLO 320 DM does form monolayer but floating cells were present, HL-60 was suspension culture. The culture flasks were checked for confluency before making it single cell. Flasks were examined under the inverted microscope (NIKON, Japan) for its glowing appearance, if contaminated; the

media becomes opaque, cell death becomes evident by opaque blackish appearance. The following was the method adopted to prepare the single cell suspension of three different cell lines:

1. The confluent flask (upto 90% confluency) of MCF-7 was selected. The spent media was then discarded and washed with 4-5 ml of PBS. About 2ml of Trypsin: EDTA solution was added, rinsed and discarded. About 2-3 drops of Trypsin: EDTA solution was then added and incubated in 37ºC for 2-3 minutes. After incubation, flask was gently tapped and visualize under inverted microscope for disruption of the monolayer. After which it was neutralized with DMEM containing FCS (10%) and triturated to obtain single cell suspension.
2. COLO 320 DM partially forms monolayer (meaning that flasks upon reaching 80-90% confluency, cells which are found adhered to the base and also in the media too). Thus the cell were scraped and triturated to disrupt the partial monolayer. The homogenous cell suspension was pelleted at 1500 rpm at 4ºC for 10 min. The supernatant was discarded. The pellet was dispersed in suitable volume of RPMI 1640 containing FCS (10%) and triturated to obtain single cell suspension.
3. HL-60 being a suspension culture does not require Trypsin or scraping. The media containing cells of confluent flask was completely dispensed to centrifuge tube and was centrifuged at 1500 rpm at 4ºC for 10 min. The pellet obtained was dispersed in suitable volume of RPMI 1640 containing FCS (10%) and triturated gently to obtain single cell suspension.

Cell Viability Assay by MTT reduction

The cells were plated in a 96-well plate (TARSONS, India) at density of $5x10^4$cells/well. The next day, different concentration of curcuminoids (prepared in suitable media for specific cell line as mentioned above) was added and was incubated for 24h, and 48h.

MTT (Sigma, USA) was prepared at concentration of 5mg/ml in PBS (Himedia, India) and filter sterilized through 0.2µm filter, and stored in sterile container at 4ºC till use. After the incubation was over 30µl of MTT was added to each well and incubated at 37ºC. After 4hr, 100 µl of SDS (10% in 0.01% HCl) was added and incubation was continued upto next day at 37ºC. Thus facilitates the formazan granules formed dissolve evenly in a homogenous manner. Readings were taken from plate reader (ANTHOS HT-II, Austria), using optical density at 540nm/reference filter 690nm.

Cell Proliferation Assay

The cells were plated at a density of $1x10^4$ cells/well in 24-well plate (Greiner bio-one, frickenhausen). Different concentration of curcuminoids (prepared in suitable media for specific cell line as mentioned above) was added to next day. After 24h of drug addition, single cell suspension was prepared. A Neubauer chamber used to count the cell number; a trypan blue exclusion test was carried out to determine the cell viability. All of the experiments were carried out in duplicate at the three different passages of the cell lines.

Data analysis

The data was analyzed by Graph Pad Prism-4 software. Cell Viability data was analyzed by linear regression to calculate IC_{50} and the data was expressed as mean + S.D. Cell proliferation data was analyzed by ANOVA followed by bonferroni test, and $p< 0.01, 0.001$ was considered significant.

RESULTS

1. Effect of curcuminoids on cell viability by MTT reduction:

The IC_{50} of curcumin was least when compared to Demethoxycurcumin and Bis-demethoxycurcumin in all the three lines:

(A) In MCF-7, it was approximately two fold and five fold with Demethoxycurcumin and Bis-demethoxycurcumin respectively when compared to Curcumin at both 24h and 48h (Table 3.1).

(B) In HL-60 the IC_{50} of curcumin was least when compared to Demethoxycurcumin and Bis-demethoxycurcumin. It was estimated that the IC_{50} of Bis-demethoxycurcumin was approximately eight fold higher and two fold higher in the case of Demethoxycurcumin compared to curcumin at 24 h treatment (Table 3.1).

(C) The IC_{50} of curcumin in COLO 320 DM was least when compared to Demethoxycurcumin and Bis-demethoxycurcumin. It was estimated that the IC_{50} of Bis-demethoxycurcumin was approximately five fold higher at 24 h treatment (Table 3.1).

Morphological Observations

A concentration above the IC_{50} value, toxic changes were observed. The cells were rounded and opaque at higher concentrations of curcumin. However, at sub-toxic levels, it was observed that the cells remained sub-confluent without any morphological changes (Fig 3.1B; Fig 3.2B; Fig 3.3B) with respect to control (Fig 3.1A; Fig 3.2A; Fig 3.3A) respectively.

Cell Line	**MCF-7**		**HL-60**		**COLO 320 DM**	
Drug/Time	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
Curcumin	1.91 + 0.21	1.75 + 0.39	0.57 + 0.02	0.92 + 0.03	0.94 + 0.03	1.12 + 0.05
Demethoxycurcumin	2.81 + 0.18	3.25 + 0.5	1.07 + 0.05	1.10 + 0.03	1.6 + 0.02	1.60 + 0.02
Bis-demethoxycurcumin	10.38 + 0.59	10.22 + 0.1	4.72 + 0.05	3.14 + 0.01	5.57 + 0.66	5.57 + 0.66

Table 3.1: Effect of curcuminoids on Cell Viability. The table depicts the values IC_{50} (µg/ml) of curcuminoids on three different cell lines at two different time points. Values are mean + S.D. of three experiments performed in duplicates at the three different passages of the cell lines.

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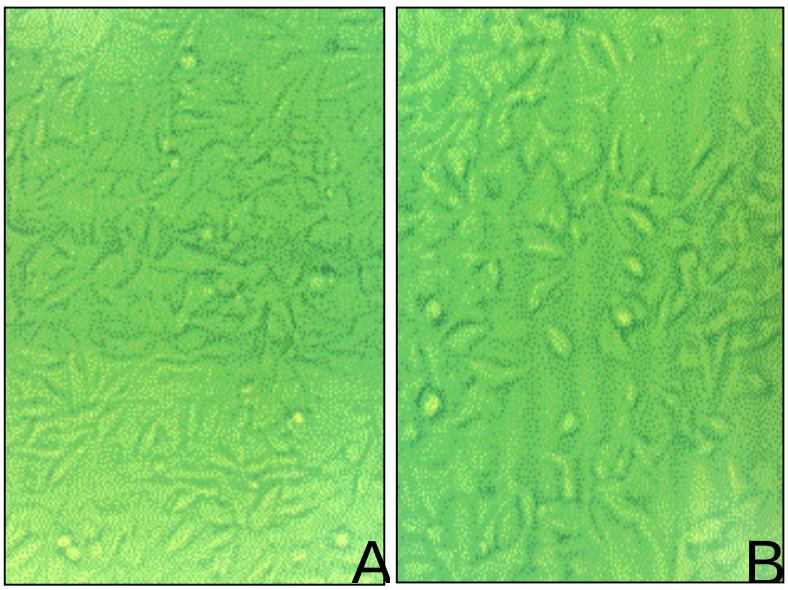
Fig. 3.1: Effect of Curcumin on proliferation inhibition in MCF-7



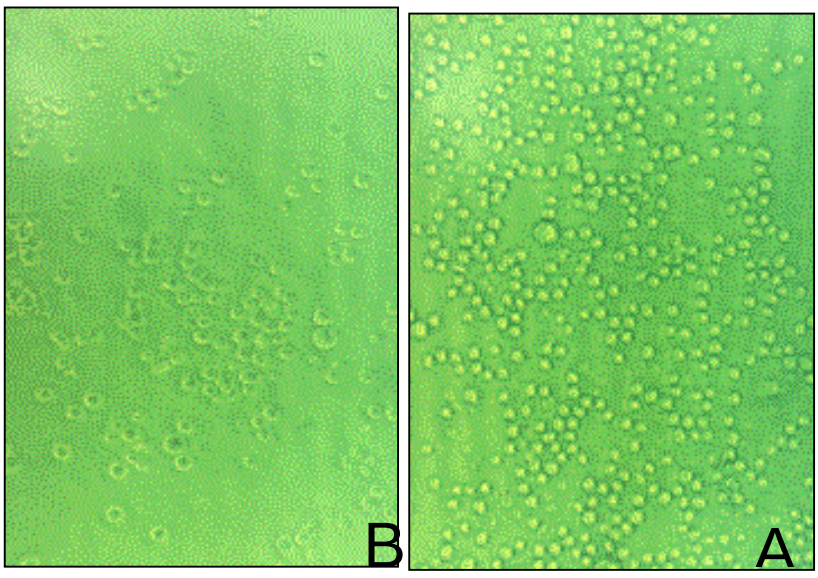


Fig. 3.2: Effect of Curcumin on proliferation inhibition in HL-60

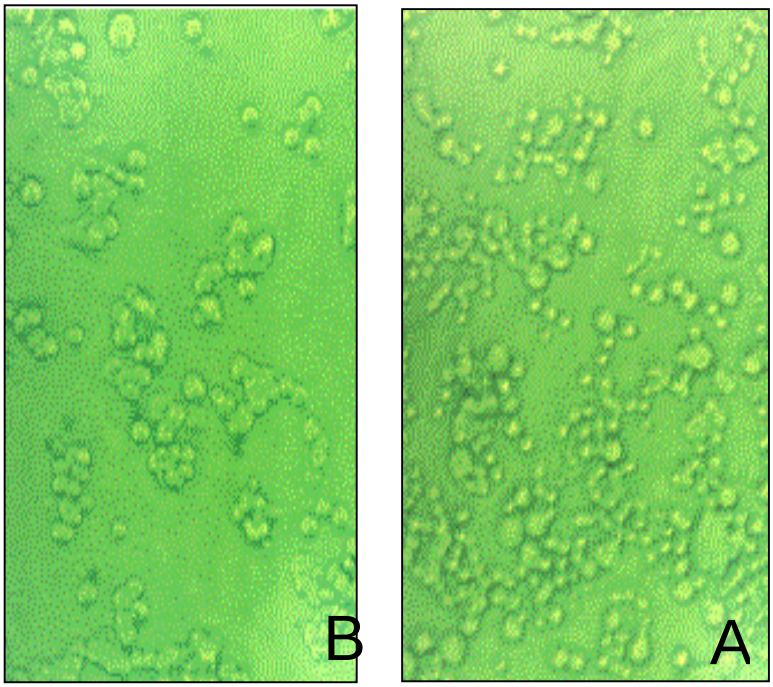


Fig. 3.3: Effect of Curcumin on proliferation inhibition in COLO 320 DM

2. Effect of Curcuminoids on Cell proliferation

(A) **Effect of Curcumin on Cell proliferation**: It was observed that Curcumin inhibited cell proliferation in dose dependent manner. In MCF-7, 50% viability was observed at a concentration of 0.8 µg/ml and statistically, at 0.4µg/ml; p<*0.01* and 0.7µg/ml, 1.5µg/ml; *p<0.001* as compared to control. In HL-60, 50% viability was observed at a concentration of 0.5 µg/ml and statistically, at 0.4µg/ml, 0.7µg/ml; *p<0.001* as compared to control. In COLO 320 DM, 50% viability was observed at a concentration of 0.8 µg/ml and statistically, at 0.4µg/ml; *p<0.01* and 0.7µg/ml, 1.5µg/ml; *p<0.001* as compared to control (Fig 3.4).

(B) **Effect of demethoxy curcumin on cell proliferation:** It was observed that demethoxy curcumin inhibited cell proliferation in dose dependent manner in MCF-7 and HL-60. In MCF-7, 50% viability was observed at a concentration of 1.2µg/ml and statistically, at 0.5 µg/ml; *p<0.01* and 1.0µg/ml, 2.0µg/ml; *p<0.001* as compared to control. In HL-60, 50% viability was observed at a concentration of 0.4 µg/ml and statistically, at 0.1µg/ml; *p<0.01* and 0.5µg/ml, 1µg/ml, 2µg/ml; *p<0.001* as compared to control. In COLO 320 DM, 50% viability was observed at a concentration of 1.02µg/ml and statistically, at 0.5µg/ml, 1.0µg/ml, and 2.0µg/ml; *p<0.001* as compared to control (Fig 3.5).

Figure. 3.4: Effect of Curcumin on cell viability

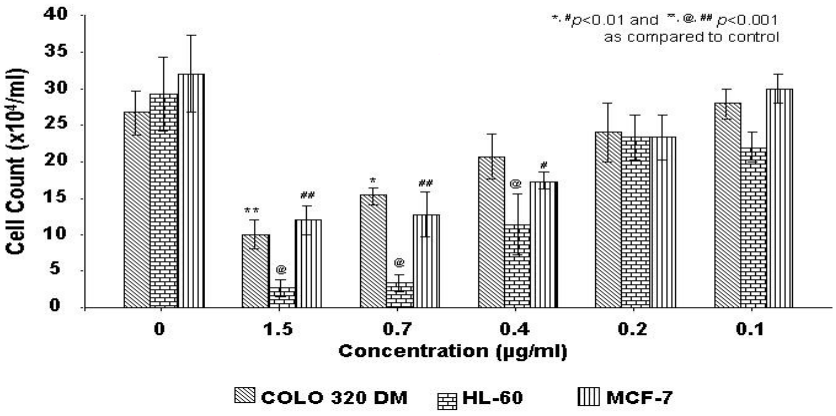
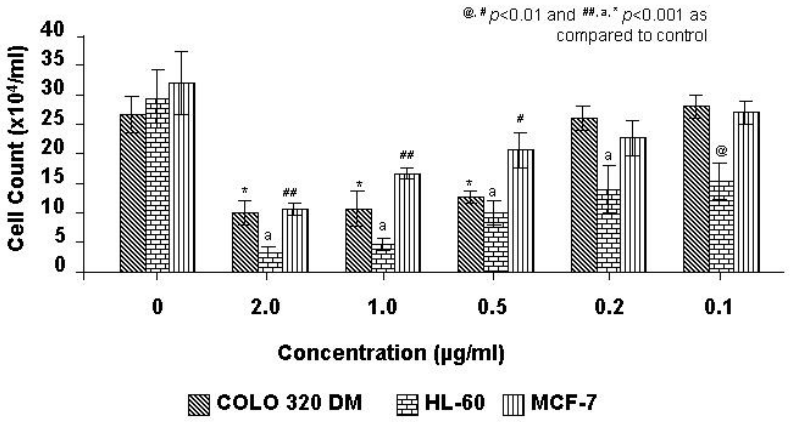
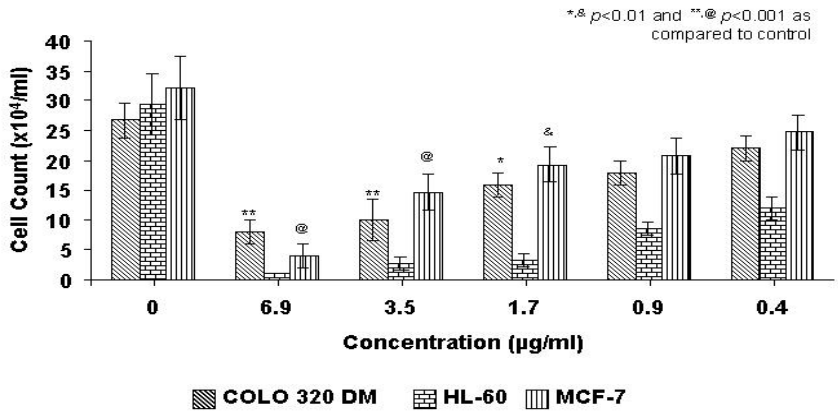


Figure. 3.5: Effect of De-methoxy Curcumin on cell viability



(C) **Effect of Bis-demethoxy curcumin on cell proliferation:** It was observed that Bis-demethoxy curcumin inhibited cell proliferation in dose dependent manner in MCF-7 and COLO 320 DM. In MCF-7, 50% viability was observed at a concentration of 2.9µg/ml and statistically, at 1.7µg/ml; *p<0.01* and 3.5µg/ml, 6.9µg/ml; *p<0.001* as compared to control. In HL-60, 50% viability was observed at a concentration of 0.3µg/ml. In COLO 320 DM, 50% viability was observed at a concentration of 3.2µg/ml and statistically, at 1.7µg/ml; *p<0.01* and 3.5µg/ml, 6.9µg/ml; *p<0.001* as compared to control (Fig 3.6).

Figure. 3.6: Effect of Bis-demethoxy Curcumin on cell viability



DISCUSSION

The concept that cancer can be prevented or certain diet-derived substances can postpone its onset has been currently eliciting considerable interest. Chemoprevention thought to be a promising anti-cancer approach with reduced secondary effects in comparison to classical chemotherapy. Three decades of research have revealed that cancer will be easier to prevent than to treat and that consumption of certain fruits and vegetables can reduce the risk of cancer. Chemotherapy aims to destroy cancer after it appears, and chemoprevention involves the abrogation or delay in the onset of cancer. Phytochemicals which are used in chemoprevention include genistein, resveratrol, di-allylsulfide, S-allylcysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, betulinic acid, flavopiridol, silymarin, anethol, catechins and eugenol. Recent reports have

shown that these phytochemicals also can reverse chemoresistance. Because of their pharmacological safety, these agents can be used alone to prevent cancer and in combination with chemotherapy to treat cancer (Aggarwal et al., 2004).

Curcumin in the spice turmeric, genistein in soya, and catechins in tea have tumor-suppressing properties in rodent models of carcinogenesis, and they interfere with cellular process involved in tumor promotion and progression (Gescher et al., 2001). Curcumin was also described as an anti-tumoral, anti-oxidant and anti-inflammatory agent capable of inducing apoptosis in numerous cellular systems (Duvorix, 2005). Commercial curcumin, pure curcumin and demethoxycurcumin were shown to be equipotent as inhibitors of 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced tumor promotion in mouse skin, where bis-demethoxycurcumin is somewhat less active (Huang et al., 1997). It was demonstrated that the substitution pattern on the aromatic moiety was especially crucial for activity (Gafner et al., 2004).

The cytotoxicity of bis-demethoxycurcumin was least when compared to curcumin and demethoxycurcumin on all the three lines considered in the present study. However, cytotoxicity of curcumin and demethoxycurcumin was comparable with marginal difference. It was reported previously that bis-demethoxycurcumin was the most cytotoxic to Ehlrich ascites tumor in mice when compared to other two derivatives (Ruby et al., 1995).

It was observed that cytotoxicity of curcuminoids was the most on HL-60 when compared with MCF-7 and COLO 320 DM. Curcumin was highly cytotoxic on HL-60 (0.57+0.02 μg/ml) when compared to COLO 320 DM (0.94+0.03 μg/ml) and MCF-7 (1.91+0.21 μg/ml). This shows curcumin can be a potent proliferative inhibitor to HL-60. Substantiating the above findings with dose response studies which were indicated that curcumin to be the most cytotoxic when compared to other phenolic compounds (Curcumin, yakuchinone B, resveratrol and capsaicin) towards HL-60, chronic myeloblastic leukemia (K-562) and MCF-7, however, did not show much activity in cervical epithelial carcinoma (HeLa) cells (Roy et al., 2002).

In the present study, three naturally occurring curcuminoid compounds were evaluated for its anti-proliferative activity in three different cell lines. The antiproliferative activity of curcumin and demethoxycurcumin were comparable in MCF-7 and COLO 320 DM. However, bis-demethoxycurcumin was marginally high to MCF-7 (2.9 μg/ml) as compared to COLO 320 DM (3.2 μg/ml). It was shown that curcuminoids was more potent proliferative inhibitors than cyclocurcumin on MCF-7 (Simon et al., 1998).

Curcumin (0.8 μg/ml) was three fold potent than bis-demethoxycurcumin (2.9 μg/ml) on MCF-7. It was reported that curcumin inhibited campothecin-, mechlorethamine-, and doxorubicin-induced apoptosis of MCF-7, MDA-MB-231 and BT-474 human breast cancer cells by upto 70%. (Somasundaram et al., 2002).

All the three derivatives were equipotently antiproliferative on HL-60. It was reported that curcumin inhibited the proliferation of K562 cells and the inhibitory effect was correlated with down regulation of abundance of p21O (bcr/abl) (Wu et al., 2003).

In similarity to MCF-7, the activity of curcumin and bis-demethoxycurcumin on COLO 320 DM was comparably same. Age plays a significant role in the efficacy of chemoprevention of colon cancer by curcumin (Kwon et al., 2004). Curcumin could be useful in the chemoprevention of human instestinal malignancies related to APC mutations. It was reported that curcumin would be in advantage over nonsteriodal anti-inflammmatory drugs and its ability to decrease instestinal bleeding linked to adenoma maturation (Perkin et al., 2002). It was observed that sunlinda, celecoxib, curcumin and nifedipine displayed dose- and time-dependent antiproliferative activities. However, celecoxib and curcumin were most effective anti-proliferative and apoptosis-inducing agent respectively tested colorectal and endometrial cancer cell lines (Wei et al., 2004).

The combined invitro assay studies for cell proliferation (MTT assay) and apoptosis (DNA fragmentation) in human colorectal cancer cells, a number of naturally occurring chemopreventive agents such as curcumin quercetin, auraptene, 1'-acetoxychavicl acetate (ACA) and indole-3-carbinol were shown to generate apoptosis as well as to inhibit cell proliferation. The results suggested that such invitro short-term assay will be useful for detection of new agents for cancer prevention (Mori et al., 2001).

FINDINGS, CONCLUSION AND SUGGESTION FOR FURTHER RESEARCH

The results of present study suggest that the proliferation inhibition potential of curcumin was higher and followed by demethoxy curcumin and bis-demethoxy curcumin. Among the three cell lines considered in this study, curcumin was shown to be most effective on HL-60 followed by COLO 320 DM and MCF-7.

From the above results, it can be concluded that curcumin as a potent anti-proliferative agent, when compared to demethoxy curcumin and bis-demethoxy curcumin against HL-60, COLO 320 DM and MCF-7.

It can be suggested that further research may be intended for
(a) Evaluating the potency of curcuminoids on different cancer cell lines
(b) Evaluating the anti-proliferative property of newer analogs of curcumin in cancer cell lines (invitro model) and extending these results to animal models.

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