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Potentiation of Curcumin and Wnt/β-catenin signaling in breast cancer

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SUMMARY

Breast cancer is the commonest malignancy among females worldwide including India. In recent years, the use of natural dietary product in breast cancer treatment has attracted great interest. Curcumin is the active component of turmeric which possesses anti-oxidant, anti-inflammatory, anti-ageing and anti-carcinogenic properties against wide range of diseases. Both *in vitro* and *in vivo*, studies have revealed that curcumin targets various genes involved in vital cellular processes. The curcumin mediated inhibition of human breast cell growth is arbitrated by certain signaling cascades including modulation of Wnt/β-catenin pathway. This signaling is aberrantly activated in breast cancer and found to be associated with its aggressive phenotype. Curcumin inhibits the expression of various component of this signaling in order to suppress the breast tumor growth. Current review summarizes the studies revealing the preventive and therapeutic potential of curcumin with an emphasis on its multi-targeted biological and molecular effects towards Wnt/β-catenin signaling in a breast carcinoma model.

Keywords: Breast cancer, curcumin, Wnt/β-catenin pathway, therapeutic modulation.

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INTRODUCTION

The malignancy of breast is often associated with high female mortality worldwide (1) and is second most common cancer among Indian females (2). In India breast cancer is highly frequent in urban Indians as compared with rural ones. This significant variation in incidence of breast cancer may be associated with the lifestyle related and reproductive/ hormonal related risk factors. The most important reproductive factors that enhance the risk of breast cancer include a long menstrual history, nulliparity, recent use of postmenopausal hormone therapy or oral contraceptives and late conception. Alcohol consumption also increases the risk of breast cancer (3). Among all these predisposing risk factors family history as well as mutations in BRCA-1 and BRCA-2 genes may also play a significant role in development of breast cancer (4). Breast cancer showed considerable heterogenecity with regards to its biological, histological and clinical behaviour (5). The criterion of breast cancer sub typing is primarily based on the presence or absence of estrogen receptor (ER) or the progesterone receptor (PR), or the up regulation of the human epidermal growth factor receptor 2 (HER2) (6,7). The cases which show positivity for all these receptors are considered as triple positive and those that show negativity are considered as triple negative breast cancers. In India, mostly the breast cancers are ER/PR negative and are characterized by aggressive clinicopathological features such as higher tumor size, tumor grade and a higher rate of lymph node positivity (8). Hence, due to the involvement of these factors in the process of development of breast cancer it has been envisaged that alteration of cellular proteins may play a major role, probably through the activation of several signaling pathways. The over activation of cellular signaling, then results in tumor expansion, cell proliferation, invasion and metastasis (9).

The breast cancers are often diagnosed in advanced stages and the biology of these tumors is not well understood till date. Despite the availability of standard protocol for breast cancer treatment like, chemotherapy, surgery or targeted therapy, the patient's outcome and response to therapy remains inconstant. The use of chemotherapeutic agents such as cisplatin, paclitaxel, carboplatin, bevacizumab, doxorubicin, cyclophosphamide, docetaxel, and epirubicin (10, 11) is always associated with their cytotoxic effects which again affect the health of patients. Hence, there is an urgent need to identify certain components that can be used in breast cancer treatment with lesser side effects. In this respect, identification of certain natural products with their targeted anti-cancerous properties can serve as a better therapeutic avenue for the treatment of breast cancer.

Curcumin :

Curcumin [1,7-bis(4-hydroxy 3-methoxy phenyl)-1,6- heptadiene-3,5-dione, Fig. (1)] is a bioactive component

Fig-1: Solid turmeric containing yellow coloured curcuminoids. The chemical structure of curcumin (Diferuloylmethane), IUPAC name: (1E,6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3,5-dione; MF: C21H20O6; MW: 368.37.

of Indian spice i.e. turmeric. It is used as a colouring agent in many Indian recipes. It has also been the important component of Indian Ayurvedic medicine and is used traditionally to treat wounds and respiratory disorders (12, 13).Biologically it is yellow coloured curcuminoid, a polyphenol derivative of rhizome of Curcuma longa (ginger family, Zingeberaceae) that makes approx. 2-6% of turmeric (11). It was first isolated in 1815, obtained in crystalline form in 1870, and identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E)ordiferuloyl methane (9, 11). Chemically, curcumin depicts a bis-α, β-unsaturated β-diketone (commonly called diferuloyl methane) structure that exhibits ketoenol tautomerism, having a predominant keto form in acidic and neutral solutions and a stable enol form in alkaline media (14).

Properties of Curcumin :

It has been extensively reviewed that curcumin can fight against wide range of diseases, due to its anti-inflammatory, anti-oxidant, anti-ageing, antimicrobial and insecticidal properties (11). Therefore, number of studied have been carried out to establish its biological as well as clinical role in various chronic diseases, including diabetes, cardiac disorders, arthritis, obesity, depression, Alzimer's and autoimmune diseases (15-20).

Anti-tumorogenic property of Curcumin :

In addition to various biological properties, curcumin has also been started using therapeutically due to its unique anti-cancerous property. The anti-tumorigenic effect of curcumin has been analysed in number of human cancer from last 50 years, which showed that curcumin affects the cancer at its initiation as well as progression stage. The in-vitro studies which were carried out on various cancer cell lines proved that curcumin plays anti-tumoral, chemo-preventive as well as chemo-radio sensibilizing role in various primary as well as recurrent malignancies. Overall, curcumin has a potential role not only to prevent but also to cure cancer (9,

21-23). The anti-cancerous potential of curcumin with respect to its tumor growth suppressive activity have been reviewed in almost all kind of cancer including, malignancies of digestive system (esophageal, gastric, intestinal, colorectal, hepatic, pancreatic), urogenital system (kidney, bladder, prostate), reproductive system (cervix, ovary), haematological system (leukaemia, lymphoma, multiple myeloma). In addition, curcumin is also found to be effective in pulmonary, thymic, brain and bone cancers (24).

It has been observed that curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancer through interacting with multiple proteins (25). The underlying molecular mechanism of effect of curcumin may be due to its ability to target the various cellular signaling and their components. The precise mechanisms by which curcumin selectively kills cancer cells is not fully disclosed; however it is proposed that the anti-cancer potential of curcumin may depend on its ability to suppress the proliferation of cancer stem/progenitor cells and their progenies, through multiple molecular mechanisms (13). Curcumin modulates various oncogenic signaling pathways i.e. inflammatory pathways (TNFα, IL-1, IL-6), protein kinases pathway (mTOR, JNK, MAPK, AKT/PKB, Cox-2), cell proliferation and survival pathways (CDKs, Cyclin E, Cyclin D, c-Myc, c-Jun, Bcl-2, Bcl-xl, cFILP, XIAP, c-IAP1), mitochondrial and caspase activation pathway (Caspase-8, 9, 3) (26). Apart from this, certain receptors like EGFR/erbB1, erbB2/HER2, IGF-1R, PPAR-γ, SHH/GLIs are also the potential target for curcumin activity (9, 27-29). It also modulates the metabolizing biotrans forming enzymes (Phase-I and Phase-II) which are responsible for activation of pro-carcinogens during carcinogenic process. Studies have shown that curcumin inactivates cytochrome P450 (CYP) activity and up-regulates GSTs (30-33).

Curcumin in Breast Cancer:

In preclinical studies, the effect of curcumin have been extensively analysed in wide range of malignant, metastatic, ER/PR/HER2$^{-/+}$ breast cancer cell lines and it has been observed that curcumin effectively counteract their growth, thereby suggesting a multifaceted role of curcumin in breast malignancy. Verma et al, 2003, studied the effect of curcumin on MCF-7 (ER+/PR+) breast cancer cell lines and suggested that curcumin exerts the anti-proliferative impact on breast cancer cells through inhibition of PS2 and transforming growth factor-β (TGF-β) gene expression. Furthermore they also reported that curcumin effectively breaks the copper-protein moieties of cells thereby reducing the growth of MCF-7 (ER+), T47D and MDA-MB-231 (ER-) cells (34). Curcumin exerts anti proliferative action mainly by interfering at the various steps of cell cycle. On experiments on MCF-7 and MDA-MB-231 breast cancer cell lines, Prasad et al, 2009 showed that, curcumin inhibits proliferation of breast cancer cells by inducing G2/M cell cycle arrest, leading to apoptotic cell death through p53

dependent manner (35). In addition, Chiu et al, 2009; showed that curcumin inhibits cell proliferation as well as migration by trans-activation of Bax/Bcl-2 proteins ratio and inactivation of NF-Kβp65 in breast cancer MDA-MB-231 cells (36). Altenburg et al, 2011 studied the effect of curcumin on SK-BR-3 (ER-/PR-/HER2+) breast cancer cell lines and observed that the breast cells supplemented with DHA enhance their curcumin uptake and thereby increased the cellular content of peroxisome proliferator-activated receptor gamma (PPAR-γ) and phospho-p53 (37).

Apart from these studies, there are reports which further suggest that curcumin also inhibits the breast tumor angiogenesis by targeting the pro-angiogenic proteins. It has been observed by Chakraborty et al, 2008, that curcumin suppresses tumor angiogenesis in breast cells by preventing osteopoitin mediated VEGF expression (38). Similar to these findings, Carroll et al, 2008 also reported that curcumin helps in the reduction of angiogenesis in T47-D (ER+) breast cancer cell line by reducing medroxy progesterone acetate (MPA) induced expression of VEGF. Furthermore they suggested that, the level of VEGF was not affected in progesterone or progestin exposed cells by curcumin through MPA (39). In another study, curcumin showed the inhibitory effect towards α6β4 integrin signaling in breast cancer cells by sub-cellular localization of α6β4, resulting in the loss of association between epidermal growth factor receptor (EGFR) and Akt receptors (40).

Evasion of apoptosis by tumor cells is the hallmark characteristic of cancer. To bring the apoptosis of neoplastic cell has always been a pre-requisite for the suppression of tumor growth. Number of experiments demonstrated that curcumin induces apoptosis and inhibits cell growth through modulating apoptotic proteins of intrinsic as well as extrinsic apoptotic pathways in cancer cells. Curcumin triggers the apoptosis of breast cancer cells by down regulating IGF-1 system molecule (important for breast cancer development) i.e. IGF-1 (41). In addition, curcumin also suppresses the microtubule instability and stimulates the activation of mitotic checkpoints for the induction of apoptosis in MCF-7 (ER+/PR+) breast cancer cells (42). In triple negative breast cancer cells it induces DNA damage and brings the cytoplasmic preservation of breast cancer type 1 susceptibility protein (BRCA1) (43). Curcumin also induces the expression of miR-15a and miR-16 by epigenetic regulation thereby reducing the Bcl-2 protein expression in breast cancer cells (44). In another study, it was observed that curcumin low doses induces p27 expression and inhibits Skp2, Her2, Cyclin E and CDKs activity in MDA-MB-231 (ER-/PR-/low HER2) cells, suggesting its probable involvement in the growth inhibition. It was also reported that curcumin in high doses was able to induce apoptotic death in MDA-MB-231 (ER-/PR-/low HER2) cells, due to the cleavage of PARP and activation of caspase 3 (45).

Cancer cells have the capability to

evade the host immune system by secreting multivesicular bodies, called exosomes (9). Curcumin has been shown to reverse the inhibitory action of exosomes towards Jak3- mediated activation of Stat5 for natural killer (NK) cells functions in breast cancers (46). Purified exosomes from TS/A breast cancer cells inhibits IL-2-induced natural killer (NK) cell cytotoxicity. The dietary polyphenol curcumin partially reverse the tumor exosome-mediated inhibition of NK cell activation, which is mediated through the impairment of the ubiquitin-proteasome system (47). Apart from that, in line with various *in vitro* studies, some *in vivo* studies were also carried out to delineate the role of curcumin in breast cancer. The overall activity of curcumin with respect to its targets in breast cancer is summarized in **Table-1**.

Table 1: *In vitro* studies depicting anti-cancerous role of curcumin in breast cancer

Breast cancer cell lines	Effect of curcumin on cancer cells	Proteins targeted	References
MCF-7 (ER/PR+), T47D and MDA-MB-231(ER-)	Anti-proliferative, inhibition of cell growth	↓PS2,↓ TGF-β	Verma *et al*, 2003
MCF-7 and MDA-MB-231	Inhibits cell proliferation and induced apoptosis	↑ Maspin, ↓ Bcl-2,↑ p53	Prasad *et al*, 2009
BT-20, T-47D, SKBR3 and MCF- 7	Arrest cell cycle at G2/S phase and inhibits cell proliferation	↓ODC	Mehta *et al,* 1997
MDA-MB-231	Inhibition of cell proliferation and migration	↑Bax, Bcl-2 ratio; ↓ NF-Kβp65	Chiu *et al*, 2009
SK-BR-3, MDA-MB-231, MDAMB- 361, MCF7 and MCF10AT	Inhibition of cell proliferation, cell cycle progression and metastasis, induction of cell cycle arrest	↑ PPARg, ↑phospho-p53	Altenburg *et al*, 2011
Breast cancer tissue cell culture	Abrogation of osteopontin-induced VEGF expression	↓VEGF	Chakraborty *et al*, 2008
T47-D	reduces medroxyprogesterone acetate (MPA) induced expression of VEGF	↓ VEGF	Carroll *et al*, 2008
MDA-MB-231	inhibitory effect toward α6β4 signaling	↓ α6β4, ↓EGFR/AKT	Soung *et al*, 2011
MCF-7	Induces apoptosis	↓IGF-1,↓ IGFBP-3, IGF-1R tyrosine ↓Kinase	Xia *et al*, 2007
MCF-7	Induces apoptosis	↓microtubule instability	Banergee et *al*,2010
MCF-7 (ER+/PR+)	Induces apoptosis	↑ BRCA-1	Rowe *et al*, 2009
MCF-7	Epigenetics changes and apoptosis	↑ miR-15a & miR-16; Bcl-2	Yang *et al,* 2010
MDA-MB-231/Her-2	Repressed cell proliferation, induced G1 arrest and triggered apoptosis	↑p27,↓Skp2, ↓Her2, ↓ Cyclin E, ↓CDKs,↓ cleavage of PARP, ↑caspase 3	Sun *et al.*, 2012

Abbreviations: PS2, an estrogen-induced RNA messenger; TGF-β, transforming growth factor-β, Bcl-2, B cell lymphoma-2; ODC, Ornithine decarboxylase; Bax, BCL2-associated X protein; NF-Kβp65, Nuclear factor kappa-light-chain-enhancer of activated B cells; PPARg, Proliferator-activated receptor gamma; VEGF, Vascular endothelial growth factor; EGFR, Epidermal growth factor receptor; IGF-1R, IGF-1 receptor; IGF-1, Insulin like growth factor-1; BRCA1, Breast cancer type 1 susceptibility protein; miR, micro RNA; Skp2, Sphase kinase protein 2; HER-2, Human epidermal growth factor receptor 2; CDKs, Cyclindependent kinases;BRACA-2, Breast cancer type 2; ER, Estrogen receptor; PR: Progesterone receptor; TNF, Tumor necrosis factor; IL-1, Interleukin-1; IL-6, Interleukin-6; JNK, c-Jun NH2-terminal kinase; Cox-2, Cyclooxygenase-2; SHH, Sonic hedgehog; DHA, Docosahexaenoic acid, NK, Natural Killer cells; Fzd, Frizzled; LRP, low density lipoprotein receptor-related proteins; APC, Adenomatosis polyposis coli;GSK3β, Glycogen Synthase kinase-3β; CK-I, casin kinase-1;Dvl, Deshevelled; TCF/LEF, T-cell factor/lymphoid enhancer factor; PI3K, Phosphatidylinositol-3-kinase; EMT, Epithelual Mesenchymel Trasition; PAC, 5-bis(4 hydroxy-3-methoxy benzylidene-)-N-methyl-4-piperidine; EAC, 1,7-Bis (4-hydroxy-3-ethoxyphenyl)-1,6-heptadien-3,5-dione; PLGA, polylactide-co-glycolide analog; PEG, Poly ethylene glycol

Curcumin and other signalling pathways in Breast Cancer :

Curcumin exerts its anti-tumorigenic activity towards wide range of proteins that are the important components of various cellular signaling pathways. As, cancer is a multifactorial disease, it is always impossible to target individual protein to suppress the tumor growth. Hence, targeting whole signaling pathway by inhibiting its all regulatory components may prove as a promising strategy for improvement of therapeutic value. Signaling pathways like canonical Wnt/β-catenin, Notch and Hedgehog are aberrantly activated in breast cancer and play a significant role in the disease development and progression. The effect of curcumin towards these signaling pathways has been analysed in multiple human cancers and observed that it modulates these signaling pathways by inhibiting them. Wang et al, 2006, reported that curcumin induces reduction of Notch-1 expression by activating hairy and enhancer of split (Hes)-1 and BclxL protein levels (48). Similarly, it has found that curcumin and its analog inhibit Wnt/β-catenin signaling in wide range of cancers. In breast cancer, where this signaling is aberrantly deregulated, the therapeutic intervention of this signaling could be the prime target of curcumin activity.

Wnt/β-catenin pathway :

The Wnts comprise of secreted cysteine-rich 39-46 kDa glycoproteins. Till date 19 members of Wnt family has been discovered in mammals (49). The Wnt gene family, the name is derived from the drosophila segment polarity gene-Wingless and the murine mammary virus gene Int-1 (50). They regulate key developmental processes including cell-fate determination, cellular differentiation, proliferation, motility and the establishment of primary axis of the body during vertebrate embryogenesis (51-53). In the adult, Wnt regulates

hematopoiesis, osteiogenesis, angiogenesis and adipogenesis (54, 55). Wnt proteins are differentiated into two group-canonical and non-canonical on the basis their transformation capabilities in cell lines or *in vivo*.

The canonical signaling gets activated by binding of specific Wnt proteins (e.g. Wnt-1; Wnt-3a etc) to the seven transmembrane domain Frizzled (Fzd) receptors on the cell membrane. Fzd proteins unite with their co-receptors, the low density lipoprotein receptor-related proteins (LRP5 or LRP6), to activate downstream Wnt signaling (56). In absence of activated Wnts, β-catenin (hallmark protein of the pathway) is phosphorylated by the destruction complex, i.e. protein complex containing adenomatous polyposis coli (APC), axin, glycogen synthase kinase 3β (GSK3β) and casein kinase I (CKI). Phosphorylated β-catenin is ubiquitinised with the help of β-TrCP and projects for proteolysis mediated degradation (57, 58). Binding of Wnts to receptors (Fzd and LRP5/LRP6) leads to the translocation of axin to the cell surface which binds with LRP5/LRP6, followed by phosphorylation of the cytoplasmic protein i.e. Dishevelled (Dvl) (59, 60,). These events ultimately consequence into the degradation or inhibition of the destruction complex, which in turn leads to accumulation of β-catenin in the cytoplasm and finally translocation into the nucleus, where it forms a complex with the T-cell factor/lymphoid enhancing factor (TCF/LEF) family of transcription factors, and activates transcription of Wnt

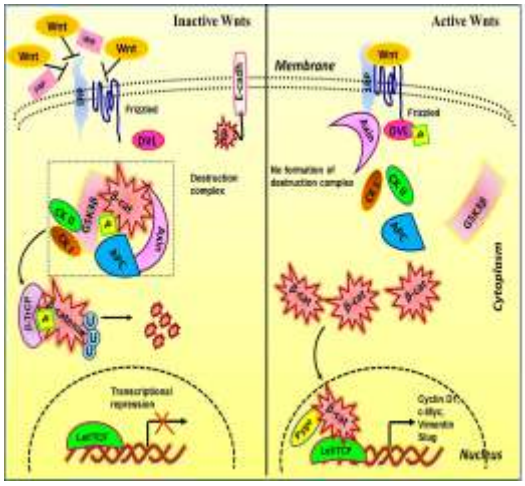

Fig- 2: In the absence of active Wnts ligand (left panel),β-catenin is associated with E-cadherin at the cell membrane. It bind to the destruction complex comprising Axin, adenomatous polyposis coli (APC)(Scaffolding proteins), glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1). The bounded β-catenin is then phosphorylated by GSK3β, ubiquitinised by beta-transducing repeat-containing protein (β-TrCP) and then degraded by proteasomal activity.
The active Wnts (right panel) bind to their transmembrane receptors Frizzled and LRPs, the formation of destruction complex is inhibited. This results in the stabilization of β-catenin in cytoplasm and translocation into nucleus. In the nucleus, it gets associated with Lef/TCF transcription factors and activates transcription of various target genes.

target genes, including c-Myc and Cyclin D1 (**Fig. 2**).

Wnt/β-catenin pathway in Breast Cancer :

Several studies have indicated the role of Wnt signaling pathways in human breast cancer. Hyper activation of the canonical Wnt/β-catenin pathway, caused by mutations in β-catenin, APC and axin, is one of the most frequent abnormalities found in other human cancers. In breast cancer, however, evidence of such mutation is sparse but β-catenin mutations have been detected in 45% cases of breast fibromatosis (61). In contrast, there is strong confirmation for elevated levels of nuclear and/or cytoplasmic β-catenin detectable by immunohistochemistry in over 50% of breast carcinomas (62-64) which correlates with the expression of its target gene Cyclin D1 and poor prognosis of breast cancer patients (64, 65).

Studies have described overexpression of Wnt proteins (Wnt2, Wnt7b, Wnt10b, Wnt13/2b and Wnt14) in breast cancer compared to normal tissues. (61, 66-70). Wnt/β-catenin pathway also showed a cross talk with phosphatidyl inositol 3-kinase/Akt and mitogen-activated protein kinase signaling pathways and two diverse receptors i.e. ER-α/EGFR (71). In addition, the various components of this pathway have also been modulated in breast cancer. The epigenetic loss of sFRP1 (negative regulator of pathway) gene has been observed in primary breast cancers and is associated with poor prognosis (72-74). Whereas, it has also been found that overexpression of DVL-1 (transducer of pathway) gene plays an important role in breast tumorigenesis through derangement of the Wnt signaling pathway (75, 65). Prasad et al 2008 reported that Wnt/β-catenin pathway components such as DVL, β-catenin and Cyclin D1 are up-regulated in invasive ductal breast carcinoma. Furthermore they also showed that the aberrant expression of these proteins were associated with advanced tumor stage of patients, hence proposed the clinical utility of these proteins in breast cancer (65). In another study, Prasad et al, 2008; showed that promoter methylation leads to loss of CDH-1 (E-cadherin) and APC genes required for the activation of Wnt/β-catenin signaling in breast cancer (76). In line with previous studies, Prasad et al, 2009 again provided the clinical evidences of modulation of expression of components of Wnt signaling like, E-cadherin, slug and GSK3β and their association with Epithelial Mesenchymel Transmission (EMT) in breast cancer (77). An APC truncation leading to β-catenin up regulation has also been shown both at cytoplasmic levels and in total cellular extracts in breast cancer cell line DU4475 (78). Transcriptional repression of APC by promoter hyper-methylation in breast cancer has been studied and found to correlate to poor prognosis in breast cancer (79-81). Moreover up-regulation of downstream targets of Wnt/β-catenin signaling viz. Cyclin D1 and c-Myc was also reported in breast cancer which was then found to be associated with progression of disease.

Activity of curcumin on modulation of Wnt/ β-catenin pathway in breast cancer :

Altered functions or levels of components of the Wnt/β-catenin pathway are associated with cancer, and other diseases (82). Therefore, Wnt/β-catenin signaling cascade increasingly attracts considerable attention of cancer researchers and pharmacologists. Dysfunctional Wnt/β-catenin signaling creates continuous transcription of many target genes supporting cell proliferation. The uncontrolled activation of transcriptional factor, β-catenin is mainly responsible for up-regulation of various proliferative genes resulting progression of disease. Hence therapeutic inhibition of this protein is the need of hour for the management of breast cancer. There are few approaches like antisense monoclonal antibodies, anti-sense oligos, RNA interference protein knockdown and chemo-sensitizers have also been developed. The main target of all these strategies is to inhibit the β-catenin activity and enhance its proteasomal degradation process, thus preventing the expression of Wnt target genes. Several reports suggest that curcumin and its analog (CHC007) are good inhibitors of β-catenin/Tcf signaling in gastric, colon, and intestinal cancer cells (9, 11, 24). Jaiswal et al 2002; suggested that curcumin suppresses the β-catenin protein activity through caspase-3-mediated blocking in intestinal cancer HCT116 cells (83). It has also been reported that curcumin controls β-catenin/TCF transcription activity by bringing down the nuclear volume of β-catenin as well as TCF-4 proteins in gastro-intestinal cancer cells (84). Moreover, the activity of Frizzled-1 receptor was also affected by curcumin (85). Curcumin also down regulates p300 in colon cancer for the attenuation of response of β-catenin to Wnt3a (86).

In human breast cancer cells, curcumin modulates β-catenin pathway by inhibiting cell proliferation and induction of apoptosis (87). Prasad et al, 2009 analysed the effect of curcumin on proliferation and modulation of components of Wnt/β-catenin signaling in breast cancer cells (MCF-7 and MDA-MB-231). They examined the effect of curcumin on breast cancer cells at different doses and its activity was detected by investigating Wnt/β-catenin signaling proteins by immune fluorescence, flow cytometry and western blotting. In these experiments, they observed that curcumin exerts a cytotoxic effect on MCF-7 cells with 50% inhibitory concentration (IC50) of 35 uM, while IC50 for MDA-MB-231 was 30 uM. Upon curcumin treatment, there was increased percentage of cells in G2/M as well as G0/G1 phases, for both the breast cancer cell lines. Furthermore, curcumin treatment decreased the levels (2 folds) of Wnt/β-catenin pathway proteins i. e. Dvl, β-catenin, Cyclin D1, and Slug. Increase in the expression of active GSK3β and E-cadherin in curcumin treated cells were also observed. From these findings, authors suggested that curcumin inhibits cell proliferation and induces cell death in these breast cancer cell lines. Furthermore, curcumin treatment reduced

the cellular level of Wnt signaling proteins in breast cancer cells thereby inducing β-catenin phosphorylation and its subsequent degradation. Overall they concluded that curcumin inhibits Wnt/β-catenin signaling by potentially inhibiting activity of its key components. One of the key mechanism that comes out from this study was curcumin mediated inhibition of Wnt/β-catenin induced EMT (88). The targeted inhibitory activity of curcumin on Wnt/β-catenin pathway proteins is illustrated in **Fig.3**

Bioavailability, pharmacokinetics and doses of curcumin :

The therapeutic ability of curcumin is usually compromised due to its low bioavailability. In various studies

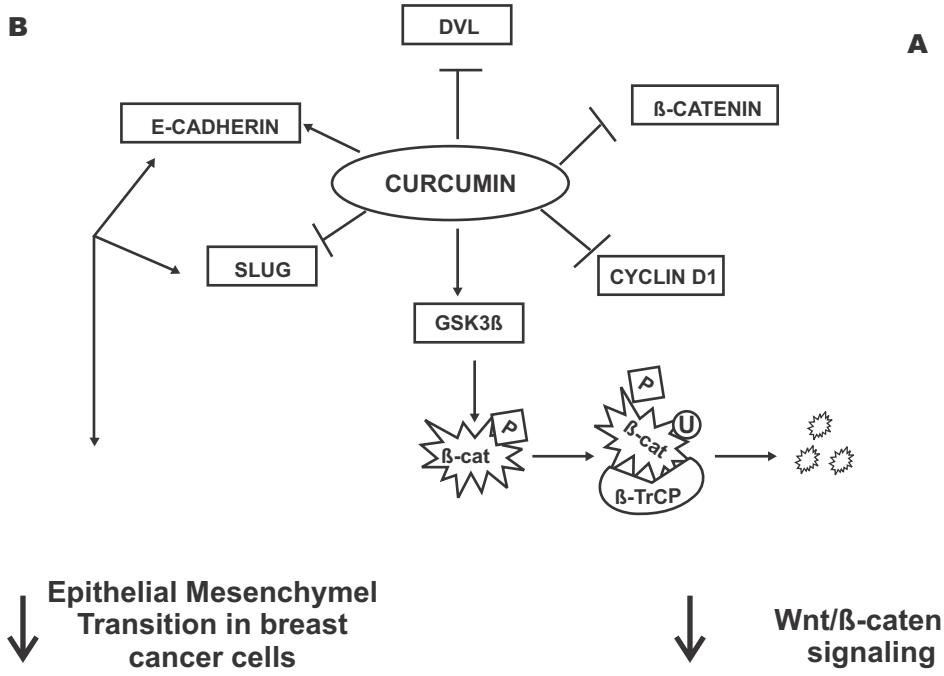

Fig-3: Schematic representation of the role of curcumin in modulation of Wnt/β-catenin signaling proteins in breast cancer cells.
Dual role of curcumin:
A. Inhibition of growth of breast tumor via modulation of Wnt/β-catenin signaling proteins. Curcumin down-regulates the expression of dishevelled (Dvl), β-catenin and Cyclin D1, but significantly retains the cellular expression of active GSK3β, leading to the phosphorylation, ubiquitination and proteasomal degradation of β-catenin.
B. Suppression of Epithelial Mesenchymel Transition through the activation of E-cadherin and inactivation of slug in breast cancer.

performed, it displayed great metabolic variability, low plasma level and poor tissue distribution. It gets poorly absorbed in the gastrointestinal tract as well as extensive metabolism through oxidation, reduction, glucuronidation and sulfation, yielding less active metabolites, and rapid elimination from the body (11, 89, 90). Some clinical studies that have been carried out on fasting volunteers and patients with precancerous lesions also revealed that the serum level of curcumin was undetectable even after administrating high dose of it (up to 2000-8,000mg) daily, which also exhibited curcumin's poor bioavailability (91 ,92). Sharma et al, 2005, suggested that the truncated systematic bioavailability of curcumin probably have been due to its hydrophobic nature, low absorption and metabolic biotransformation in intestine and liver (93). Therefore, to overcome the pharmacokinetics limitation and to improve the therapeutic value of this dietary component, various approaches like liposomal curcumin, curcumin phospholipid complex, structural analogs of curcumin, curcumin nanoparticles and curcumin conjugations have been developed and tested (94-98) in order to improve curcumin drug delivery. Among all these strategies, the efficacy of curcumin analogs and curcumin nanoparticles has been extensively tested in breast cancer. Importantly, two novel non-toxic curcumin analogues have been investigated to possess anti-breast cancer properties, namely 5-bis(4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidine(PAC) and 1,7-bis-(4-hydroxy-3-ethoxyphenyl)-1,6-heptadien-3,5-diene (EAC).PAC showed higher stability in blood, higher water solubility, greater bioavailability and bio-distribution than curcumin(9, 11). PAC displayed five times higher efficiency than curcumin and also EAC at inducing apoptosis on ER-negative-MDA-MB-231 cells via an internal mitochondrial route (9, 11). *In vivo* experimental results on MDA-MB-231 cell xenografts have proven that PAC exhibited the anti tumoral effects by inhibiting NF-kB and its downstream effectors (cyclin D1 and Bcl-2), p21WAP1, Survivin and activating the caspase cascade (99).

Curcumin encapsulated with nanoparticulate formulation based on poly lactide-co-glycolide (PLGA), along with a stabilizer polyethylene glycol (PEG)-5000 showed more potency than crude curcumin, in inducing anti-proliferative and apoptotic effects against MDA-MB-231 cells (98). Transferrin-mediated solid lipid nanoparticles (Tf-CSLN) increased photostability and enhanced its anticancer activity against MCF-7 breast cancer cells (100). Tang et al. 2010, reported that an intracellular-labile amphiphilic surfactant (- like curcumin prodrug-curcumin) conjugated with two short oligo (ethylene glycol and Curc-OEG) chains via beta-thioester bonds acted as vehicles for doxorubicin and camptothecin treatment. On delivery to drug-resistant cells, it greatly enhanced the cytotoxicity of the loaded drug in nude mice inoculated with MDA-MB-468 cells (101).

Potential risk and side effects of

curcumin :

Phase I clinical trials discovered that curcumin could be safely administered at very high doses up to 6 g per day (102). However, it has also been observed that curcumin bears the blood-thinning effect that may decline blood flow, leading to ischemic stroke (103). In addition to its anti-oxidant effect, curcumin also demonstrate pro-oxidant effect (104). Curcumin inactivated the cancer cytotoxic agent by blocking JNK pathway in breast cancer (105). The studies performed on breast cancer cells clearly showed that curcumin inhibits mechlorethamine, doxorubicin and camptothecin and induces apoptosis of these cells (106).Therefore, more importance should be given towards the establishment of efficacy of curcumin in breast cancer treatment.

Conclusion :

From the last few years, cancer researchers thoroughly examined the anti-tumorigenic effects of curcumin. Studies suggest that this polyphenol affects various pathways and signaling proteins critical for tumorigenesis. Therefore, it has potency in cancer prevention and also cancer treatment. Among the number of pathways, Wnt/β-catenin signaling is aberrantly up-regulated in breast cancer and the modulation of its key components is found to be associated with aggressive phenotype of these tumors. Therefore, therapeutic intervention of this signaling is the topic of great interest in breast cancer treatment. The use of novel curcumin synthetic analogs and nanotechnology- based formulations represented a potential alternative strategy of great clinical interest for overcoming the high metabolic instability and poor bioavailability of curcumin. Overall, curcumin and its analog needs significant research to establish its therapeutic potential with less limitation and enhanced action in breast cancer.

Conflict of interest:

Authors do not show any conflict of interest.

Authors contributions:

Experimental analysis was carried out by Dr. Chandraprakash Prasad. Poonam Jawanjal helped in acquisition of data and references as well as writing the manuscript. Dr. Gayatri Rath reviewed the results of experiments and has compiled the manuscript.

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