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INVESTIGATION OF ANTICANCER ACTIVITY ON CURCUMIN DENDRIMERIC NANOFORMULATION

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Abstract:

Curcumin (diferuloylmethane) is a polyphenol derivedfrom the plant Curcuma longa, commonly called turmeric. Extensive research over the last 50 years has indicated this polyphenol can both prevent and treat cancer. The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of tumor cells, down-regulatetranscription factors NF-κB, AP-1 and Egr-1; down-regulate theexpression of COX2, LOX, NOS, MMP-9, uPA, TNF,chemokines, cell surface adhesion molecules and cyclin D1;down-regulate growth factor receptors (such as EGFR andHER2); and inhibit the activity of c-Jun N-terminal kinase,protein tyrosine kinases and protein serine/threonine kinases.

One draw-back holding curcumin back is its insolublility in water and therefore has poor absorption and subsequently poor bioavailability.

Dendrimer, being well defined and highly branched macromolecule, their multifunctionality and specific shape have been recognized as powerful tool in drug delivery. Polyamidation (PAMAM) dendrimers consist of core dendr-termini (i.e. DAB-dendr -(NH2)64) is a diaminobutanecore dendritic poly(propylene mine) having 64 primary amine termini, also named as DAB-dendr -(PA)64, where PA stands for polyamine, and its repeating units and terminal groups potent for interaction of oppositely charged component and produces surfactant like effects by forming a nanoparticles for drug delivery. In the context of drug delivery, the use of fatty acids as permeation enhancer limits the metabolism of drug in liver when administered orally and allowed for plasma binding in blood readily. The application f this study could be sustained for oral delivery and improved absorption through gastrointestinal tract for overall enhancement in therapeutic efficacy.

Based on these reports, the present study is designed to overcome pharmacokinetic drawbacks by conjugating with dendrimer and understanding there physicochemical properties of curcumin/dendrimer/fatty acids complex system and to evaluate its suitability for sustained drug release, which is further allowed for oral drug delivery for screening as potent anticancer agents by using in vitro and in vivo model.

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INTRODUCTION:

Cancer:

According to the International Agency for Research on Cancer (IARC) the cancer is a leading cause of death worldwide which inflicted 7.6 million deaths (~13% of all deaths) only in 2008 (Nirmala et al., 2011). Colorectal, lung, female breast and prostate cancers are main contributors to such deaths in most parts of the world causing a loss of 18-50% total healthy years (Soeriomataram et al., 2012). About 47% cancer cases and 55% cancer deaths occur in less developed countries, the countries having low or medium level of human development index (HDI). The trends in major cancers indicate that cancer harms will increase to 22 million new cases each year by 2030, indicating 73% hike over year 2008 [81% in low and middle HDI countries and 69% in high and very high HDI countries]. In India 7,137 deaths out of 1,22,429 cases were due to cancer in 2010 and estimated 71% cancer death occurred in the age range of 30 to 69 years, so showing age-specific trend in cancer diseases (Dikshit et al., 2012).

Conventional drugs suffer because of their low aqueous solubility and physical stability, reduced absorption, rapid metabolism and instability under high acidic conditions. To combat these constraints intense research is focused on various sources to develop novel anti-cancer drugs. Plants have proved valuable natural anti-cancer therapy source.Balachandran and Govindrajan (2005) have reported pharmacological actions of 40 anti-cancer herbal drugs while Bhanot et al. (2011) listed 117 plants having anticancer properties. Thus, the nano-sized drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines.

Nanotechnology is a field of applied science and technology which aims to develop devices and dosage forms in the range of 1 to 100 nm. The applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems have recently been referred to as nanomedicine. The nanocarriers have been made of safe materials, including synthetic biodegradable polymers, lipids, and polysaccharides.

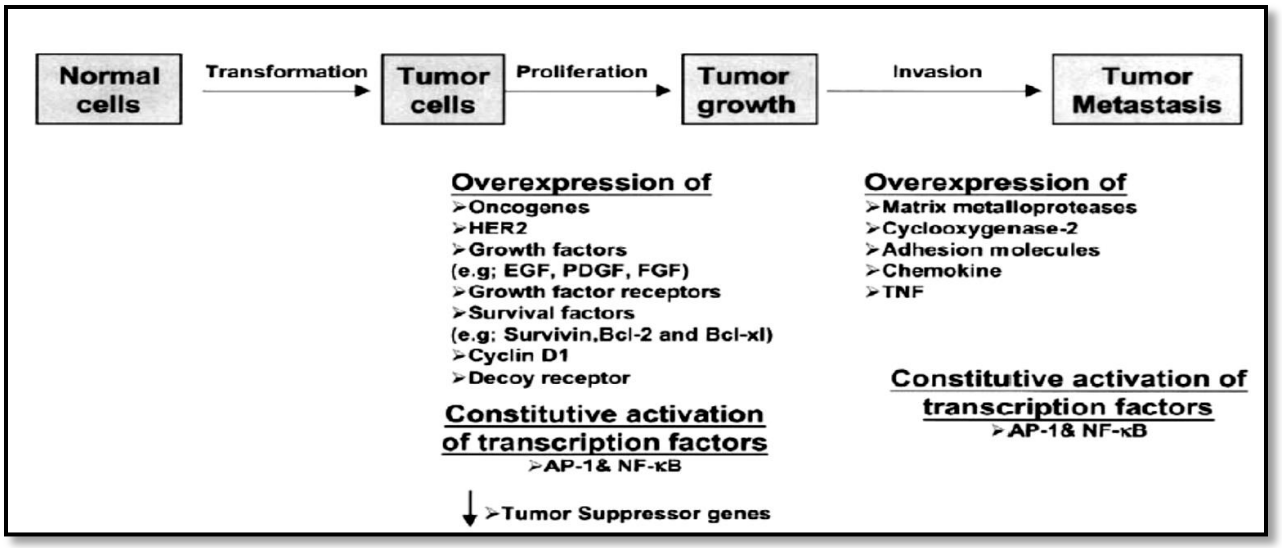

Figure 1. Biochemical Pathway of Carcinogenesis (Aggrawal et al., 2003)

Plant secondary metabolites have proved an excellent reservoir of new anti-cancer compounds and there are four major structural classes of anti-cancerous alkaloid compounds derived from plants Vinca alkaloids, Epipodophyllotoxin lignans, Taxane diterpenoids and Camptothecin quinoline. However, for safe and optimal use of anticancer herbal medicines, their pharmacokinetic must be evaluated in humans and most of such studies are focused mainly on a small number of herbal medicinesincludingcurcumin,ginseng,ginkgo,gingera ndmilk thistle(Chenet al.,2011). Despite wide adaptability of herbal drugs, pharmacy kinetic and delivery of herbal drugs require modification so as to achievesustainedDrugreleaseandincreasepatientcompl iance.Theefficacyof manyherbaldrugsisoften limited

by their unspecific site of action. In most cases (conventional dosage forms) only a meager amount of administered dose reaches the target site and bulk of administered drug gets distributed throughout the body in accordance with its physicochemical and biochemical properties such as low solubility, reduced absorption, rapid metabolism, etc. Novel drug delivery systems (NDDS) are currently under development so as to lessen drug degradation, reduce dosage and its toxicity, increase solubility and stability, and improve tissue macrophages(KumarandRai,2012a).

Cause of cancer:

Carcinogens are the any substance or agent that is capable of causing cancer-the abnormal or uncontrolled growth of new cells in any part of the body in human or animal. Such as-

1. Milk adding chemical: Several milk constituents such as vitamin D, proteins, calcium, CLA, butyrate, saturated fatty acids, and contaminant such as pesticides, estrogen, and insulin-like growth factor-1 (IGF-1) may be responsible for either a prospective or a harmful association between dairy products and cancers. Dietary fat has been reported to increase the androgen level associated with prostate cancer risk. Dairy foods and their constituents (lactose) have been hypothesized to possibly promote ovarian carcinogenesis.

2. Obesity Obesity has been linked to more aggressive characteristics of several cancers including breast and prostate cancer. The myeloid lineage cells, in the form of myeloid derived suppressor cells (MDSCs) and alternatively polarized M2 macrophages influence almost all types of cancers by regulating diverse facets of immune suppression, angiogenesis, cell proliferation, growth and metastasis. The different aspects of obesity, namely insulin resistance, increased estrogen, adiposity and low grade chronic inflammation from adipose tissue macrophages, may coalesce to promote MDSC induction and M2 macrophage polarization, there by facilitation cancer development.

3. Cigarette smoke The various carcinogenic compounds have been identified in primary and side-stream tobacco smoke. It is a complex mixture of chemicals in tobacco smoke, including 212Pb and 210Po, react covalently with DNA and produce free radicals causing oxidative damage. Cigarette smoke exhibits very significant synergistic interaction with ethanol to induce oral/pharyngeal cancers and with asbestos to induce lung cancer.

4. Alcohol Consumption The alcohol increases the risk for cancers of the oral cavity and pharynx, larynx, esophagus, and liver. The biological mechanisms of alcohol induces cancer are not fully understood but may include genotoxic effects of acetaldehyde, production of reactive oxygen or nitrogen species, changes in folate metabolism, increased estrogen concentration. The International Agency for Research on Cancer (IARC) and the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) both published comprehensive reviews of the scientific literature on alcohol have risk of cancer.

5. Hair Dye The permanent oxidant hair dyes are consisted of many chemical components including ortho- phenylenediamines(o-PD) and and its derivatives, 4-chlor-orthophenylenediamine(CL-PD) and 4-nitro-ortho-phenylenediamine. The carcinogenic o-PD and CI-PD caused Cu(II)-mediated DNA damage, including 8- oxodG formation, and antioxidant enzyme superoxide dismutase (SOD) enhanced DNA damage. This results that SOD enhanced the rate of Cu(II)-mediated autoxidation of o- PD and CI-PD, leading to enhancement of DNA damage and produced cancer.

Chronic infections The stomach ulcers are due to Helicobacter pylori. Mycoplasmas may cause chronic lung disease in newborns and chronic asthma in adults, and Chlamydia pneumonia, a recently identified common cause of acute respiratory infection .These infection agents that cause or contribute to neoplastic diseases in human.

Fertilizer the higher levels exposure of nitrates or nitrites has been associated with increased incidence of cancer in adults and possible increased incidence of brain tumors leukemia and nasopharyngeal (nose and throat) tumors in children. The U.S. EPA concluded that there was conflicting evidence in the literature as to whether exposures to nitrate or nitrites are associated with cancer in adults and in children.

Environmental factor Exposure to Ultraviolet-B-radiation (UVB,280-320nm) is known to induce basal and squamous cell skin cancer in a dose –dependent way and the depletion of stratospheric ozone has implication for increases in biologically damaging solar UVB radiation reaching the earth, s surface. In humans, arsenic is known to cause cancer of the skin, as well as cancer of the lung, bladder, liver, and kidney.

Medical drugs Some drugs used to treat cancer (e.g. , cyclophasphamide, chlorambucil, melphalan) have been shown to increase the occurrence of second cancers, including leukemia.

Genetic disorder Down syndrome and certain other genetic diseases- some diseases caused by abnormal chromosomes may increase the risk of leukemia.

Psychological stress Psychological stress activates the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, resulting in systemic

increases in cortisol and catecholamines. The effects of catecholamines are mediated by nine distinct @-adrenergic and B-adrenergic G-protein-coupled receptors, which are present on a wide range of cell types, including cancer cells.

Hormone: Estrogen used to treat symptoms of menopause and other gynecological conditions have been shown to increases the incidence of endometrial cancer and breast cancer.

Immunosuppressant The Immunosuppressant's such as cyclosporine and azathioprine are use organ transplants in patients, also are associated with increased cancer risks, especially lymphoma.

Inherited conditions Certain inherited conditions increase a person's risk of developing soft tissue sarcomas.Such as Neuro fibromattosis, Gandner syndrome, Tuberous sclerosis etc.

Pesticides These Pesticides includes that ethylene oxide, amitrole, some chlorophenoxy herbicides, DDT, dimethylhydrazine, hexachlorobenzene, hexamethyl phosphoramide, chlordecone, lead acetate, lindane, mirex, nitrofen, and toxaphene. of people with high exposures to pesticide applicators, crop duster pilots and manufacturers have found high rates of blood and lymphatic system cancers, cancers of the lip, stomach, lung, brain and prostate, as well as melanoma and other skin cancers Studies.

Helicobacter pylori infection The major infections of H.pylori cause chronic gastritis, peptic ulcers and gastric malignancies, including gastric non- cardia adenocarcinoma and mucosal- associated lymphoid tissue (MALT) lymphoma. Epidemiologically, H. pylori infects half of world's population, approximately 1% infected people will develop in to gastric cancer.

HIV infection The HIV positive people have most common cancer is Kaposi's sarcoma and high grade non-Hodgkin's lymphoma due to AIDS- defining malignancies. The Non- Hodgkin's lymphoma occurs late in the process of AIDS; Up to 10% of people with HIV will eventually develop Non Hodgkin's lymphoma. Hodgkin's disease, squamous cell carcinoma of the conjunctiva and leiomyosarcoma also appear tobe associated with HIV infection.

Ultraviolet radiation Ultraviolet(UV) radiation from the sun, sunlamps, or tanning beds causes premature aging of the skin and DNA damage that can lead to melanoma and other forms of skin cancer. The incidence of skin cancers is rapidly increasing.

Nuclear radiation Very high levels of radiation have been caused by atomic bomb explosions (such as those in Japan during World War II) and nuclear power plant accidents (such as the Chernobyl [also called Chornobyl] accident in 1986). The ionizing radiation is the radioactive substances released by atomic bombs or nuclear weapons known as "fallout". The doses of ionizing radiation received by the atomic bomb survivors in Japan resulted in increased risk of leukemia and cancers of the breast, thyroid, lung, stomach, and other organs.

Mobile Tower The mobile Tower is mainly produce radiofrequency (RF) waves, a form of energy in the electromagnetic spectrum between frequency modulation radio waves and microwaves. But radiofrequency(RF) waves are different form stronger types of radiation such as x- rays, gamma rays, and ultraviolet(UV) light, which can break the chemical bonds in DNA then cause cancer.

A GENERALIZED MODE OF ACTION OF NDDS:

In cancer treatment six major types of drug delivery system are currently in use including both orthodox medicine and natural drugs. These are:

i) Direct introduction of anticancer drug into tumor,
ii) Systemic delivery targeted to tumor,
iii) Drug delivery targeted to blood vessels of tumor,
iv) Special formulation and carriers of anticancer drugs,
v) Trans-membrane drug delivery to intracellular targets and
vi) Biological therapies.

An ideal carrier for target drug delivery system should, therefore, have three pre-requisites for their functions: a) specific target effects, b) sufficiently strong adsorptive effects for anticancer drugs to ensure that drugs are transported to the effect-relevant sites; and c) release the drugs in effect-relevant sites. General advantages of NDDS over conventional drug delivery.

NDDS act by different mechanisms; however the two most accepted explanations behind the success of passively targeted-NDDS developed for cancer treatment are:

a) Enhanced permeability and retention(EPR)effect and
b) Possession of altered pharmaco-kinetic (PK) characteristics that are distinct from the encapsulated drug.

The EPR effect is a property by which certain sizes of molecules (like nano-particles, liposome or phyto-complex drugs) tend to accumulate in Tumor tissue much more than they do in normal tissues (Patankar and Waterouse, 2012). EPR-based targeting relies on exploiting the leaky vasculature present at the tumor site (Fig. 2). The examples of EPR based NNDS systems are nano-particles of paciltaxel and Artemissia annua, liposome of camptothecin, silymarins, ampelopsin, transferosome of curcumin,

ethosome of triptolide, phyto-complex of Vaccinium myrillus, etc.

TYPES OF DRUG DELIVERY SYSTEMS:

In phyto-formulation research, developing nano-dosage forms like polymeric nano-particles, nano-capsules, liposome, phyto-complex, nanoemulsion, ethosomes, pronisomes, floating drug delivery system, micro-emulsions, etc. are advantageous as these enhance the solubility, bioavailability and stability of drug, provide protection from toxicity, enhance pharmacological activity and protect the drug from physical and chemical degradation (Saraf, 2010; Thakur et al., 2011; Kumar and Rai, 2012a).The various types of novel drug delivery approaches used are summarized in Table 1along with their advantages.

Table 1: Various novel drug delivery systems (NDDS) and their advantages

NDDS types	Description	Advantages
Nano-particles	Drug-loaded particles prepared by taking natural polymer or synthetic chemicals as the carrier, exhibits diameter of 10–1000 nm.	a) Potentially increases solubility, bioavailability, surface area, improve controlled release and enable target specificity, improved stability. b) Efficient for both hydrophilic and hydrophobic herbal drugs (Pathak and Katiyar, 2007).
Phytocomplex (patented technology)	Involve incorporation of standardized plant extracts or water soluble phyto-constituents into phospholipids to produce lipid compatible molecular complex.	a) Enhances absorption of lipid insoluble phyto-constituents and improves the absorption of active constituents. b) Chemicals bonds between phosphatidyl-choline molecule and phyto-constituent shows better stability.
Liposomes	Lipid vesicles mainly composed of one or multiple lipid bi-layers composed of mixtures of phosphatidyl- cholines with long or short hydrocarbon chains.	The technique is efficiently utilized for enhancing the therapeutic index of anti-cancer agents, either by increasing the drug concentration in tumor cells and or by decreasing the exposure in normal tissues exploiting (Sharma et al., 2006).
Emulsions	Emulsion is a biphasic system in which one phase in intimately dispersed in other phase in the form of minute droplets. In emulsion, one phase is always water or aqueous phase, and the other phase is oily liquid.	a) Well distributed due to affinity for lymph. b) Susutained release as it is packed in the inner phase and makes direct contact with body and tissues. c) Enhance stability of hydrolyzed materials, penetration of drugs to the skin and mucous, and reduces drug stimulus (Saraf, 2010).
Transfersomes	Transfersomes are made up of phospho lipids supplemented with single chain surfactant with high radius of curvature which acts as edge activators to provide vesicle elasticity and deformability (Cevc et al., 1996).	a) Easily accommodated with a wide range of solubility. b) Act as an efficient carrier for both low and high molecular weight drugs with high entrapment efficiency. c) Protect the encapsulated drug, from metabolic degradation (Prajapati et al., 2011).
Ethosomes (a novel liposome)	Ethosome are soft, malleable lipid vesicles composed mainly of phospholipids, alcohol (ethanol or isopropyl) in relatively high concentration (20-45%) and water (Rakesh and Anoop, 2012).	a) Suitable as topical or trans-dermal administration carrier; b) Have high deformability and entrapment efficiency and can penetrate through the skin or even into the blood circulation (Dayan and Touitou., 2000)
Microspheres (particle size <200 m)	Microspheres are characteristically free flowing powders consisting of synthetic biodegradable polymers/ proteins (Singh et al., 2011).	a) Can be ingested or injected and tailored b) Used as site-specific delivery of drug and in some cases even provide organ-targeted release (Saraf, 2010).
Lipoprotein	Lipoproteins are endogenous particles that transport lipids through blood to	a) Considered excellent candidates for targeted delivery of drugs to various tissues; b) Lipid

	various cell types where they are recognized and taken up via specific receptors.	encapsulation substantially extends residence time in circulation of natural drug like paclitaxel (Lacko et al., 2005).
Dendrimer	Dendrimers are nano-sized, radially symmetric molecules with well-defined, homogeneous and monodisperse structure consisting of tree-like arms or branches.	a) Combat unfavorable properties of small molecules (like insolubility) by larger characteristics of macromolecule; b) Flexible branches of dendrimers provide tailored sanctuary containing voids which provide a refuge from the outside environment. c) Paclitaxel and docetaxel (Ooya et al., 2004) as well as the anticancer agent 10-hydroxycamptothecin (Morgan et al., 2003), have been successfully encapsulated.

PLANT PROFILE:

Curcumin

Curcumin (diferuloylmethane; see Figure 2) is a natural yellow orange dye derived from the rhizome of *Curcuma longa* Linn, an East Indian plant.

Taxanomical classification:

Kingdom- Plantae
(Unranked)- Angiosperms
(Unranked)- Monocots
(Unranked)- Commelinids
Order- Zingiberales
Family- Zingiberaceae
Genus- Curcuma
Species- Curcuma longa

Fig no 1

Chemical structure

Curcumin keto formCurcumin enol form:

IUPAC name-1,7-Bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.

Physical Properties

- Molecular formula- $C_{21}H_{20}O_6$
- Molar mass- 368.38g mol^{-1}
- Appearance- Bright yellow orange powder
- Melting point-183^0C (3610F; 456K)
- It is insoluble in water and ether but is soluble in ethanol, dimethylsulfoxide and other organic solvents.

Molecular Properties and Its Metabolites:

Chemical profile:

Commercial curcumin contains three major components: curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%), together referred to as curcuminoids. The chemical structural and spectrosco[ical demonstration of curcumin is ,mentioned in table 2.

Metabolism:
Curcuminoids can avidly donate hydrogen ions and undergo nucleophilic addition also possessing other moieties with the potential to undergo biochemical modification (Priyadarshini et al., 2006) and impart the important reduction–oxidation, anti-oxidant and proton donating properties that can combat cell damage (Webner et al., 2005), the mechanisms which enable curcumin form numerous complex.

Lin et al. showed that curcumin was first biotransformed to dihydrocurcumin and tetra hydrocurcumin and that these compounds subsequently were converted to monoglucuronide conjugates (Wahlstorm and Blennow, 1978). Curcumin-glucuronide, dihydro-curcumin-glucuronide, tetrahydrocurcumin-glucuronide, and tetrahydrocurcumin are major metabolites of curcumin in rodents (Ravindranath and Chandrasekhara, 1981).Since the systemic bioavailability of curcumin is low, its pharmacological activity may bemediated, by its metabolites. THC is obtained by partial hydrogenation of curcumin; it is colorless and more hydrophilic than curcumin (Sharma et al., 2005).

Bioavailability:
Bioavailability is the amount of active molecule that reaches the target site, be it the knee joint, the nerve cell in the brain or the cancer tissue. Unfortunately, to measure the amount present at the actual target site is very expensive and labour-intensive, thus most researchers measure the amount of curcumin that is present in the blood as a surrogate marker of bioavailability.In Phase I clinical trials, dietary curcumin was shown to exhibit poor bioavailability (i.e., low levels in plasma and tissues).Potential factors that limit the bioavailability of curcumin include insolubility in water (more soluble in alkaline solutions), poor absorption, rapid metabolism and systemic elimination. Numerous approaches to increasing curcumin bioavailability have been explored, including the use of absorption factors such as piperine. Because of its stability and physical properties, pure curcumin can be vaporized or smoked, obviating the need for oral absorption factors. This ROA however carries hisgher risk of chelating iron from hemoglobin, and potentially higher risk of carcinogenicity.

Pharmacological Significance:
Curcumin has a diverse array of favourable properties including its capacity as an antioxidant, anti-inflammatory (Sugiyama et al., 1996) and anticarcinogen (Perkins et al., 2002). Furthermore, curcumin has generally been demonstrated to have low toxicity (Cheng et al., 2001) and attributed to its numerous effects on several targets such as penetration through blood–brain barrier (Tsai et al., 2011) and modulation thelevels of neurotransmitters like norepinephrine, dopamine (Blum et al., 2001),and serotonin (Xua, 2007) in the brain. However, this hydrophobic molecule displays low bioavailability (Aggrawal et al., 2007), which is a particular challenge for cancer treatment. Hence, the diverse mechanisms of curcumin in the treatment of disease continues to be an enigma, its therapeutic usefulness and cost effectiveness make it a particularly valuable compound.

Table 2. Chemo preventive properties of Curcumin

Type of cancer	Mechanism	Ref
Head and neck squamous carcinoma	suppression of phosphorylation of eIF4E and Mnk1, inhibition of the AKT/mTOR pathway	Wilken et al., 2011
Head and neck squamous carcinoma	Downregulation of cyclin D1	Aggrawal et al., 2004
Prostrate cancer cell	Downregulation of EGRF, suppresssion of intrinsic EGRF tyrosine kinase activity and inhibition- ligand inducer EGRF activity.	Dorai et al., 2000
Non-small cell lung cancer	Inhibit constitutive NF-κB activation and COX expression	Shishodia et al., 2003

Human colon adrenocarcinoma	Enhance p53 activity	Song et al., 2005
B cell lymphoma	Supress C-myc	Han et al.,1999
Prostrate and breast cancer cell	Downregulation of cyclin D1	Mukhopadhyay et al.,2002
Breast cancer	Suppression of IGF system	Xia et al.,2007
Breast cancer	Induction of cell cycle arrest at S, G2/M phase by upregulation of p21	Chiu and Su, 2009
Human epidermoid carcinoma cell	Inhibit EGFR activity	Korutla na Kumar, 1994
Colon carcinoma cell	Induction of cell cycle arrest at S,G2 and M phase	Chen et al., 1999
Human biliary cancer cell	Downregulation of cyclin D1	Prakobwong et al., 2011
Human hepatocarcinoma cell	Induction of cell cycle arrest at S, G2 and m phase	Cheng et al.,2010
Skin tumor	Supress c-fos and c-Ha-ras-activation by DMBA	Limtrakul et al.,2001

EXPERIMENTATION:

Acute toxicity study of this compound.

- **Body weight /Body temperature**
- **Food and water observation**
- **Relative and absolute organ weight**
- **Histopathological study**
- **Heamatological study**

Biochemical parameter
To investigate the Anticancer activity in vivo.
2. Statistical Analysis

MATERIAL AND METHODS:

Sprague Dawley rats, Ethenol ,HCL, PBS, Dendrimer [polyamidoamine (PAMAM)], TB Syringes.

Animals-In the present study adult healthy female Sprague Dawley rats weighting b/w 150-200 gm, were purchased from animal house of CSIR-CDRI Lucknow for the experimental studies. Animal were acclimatized to the animal house conditions for a week. Before and during the experiment, the rats were allowed free access to standard pellet diet and water. The temperature in the experimental animal room maintained at 23^0C ($+3^0$C) and relative humidity is at least 30%. The animal room was well ventilated with 12 hours light and 12 hours dark cycle, throughout the experimental period.(OECD Guide line 423, 2001).the experimental protocol used in this study was approved by the institutional animal ethical committee-[1397/ac/10 CPCSEA].

Tumors cells and reagents:
Cell line MCF-7 cell($1*10^7$ml) were provided by CSIR-CDIR Lucknow. Cells were maintained in Dulbecco, s modified Eagle medium supplemented with anticancer, L-glutamine (2 Mm) and 10% fetal calf serum (allform Life Technologies, Inc.; culture medium) and passaged every 3-4 days. After inoculation period, the tumor cells were harvested from the cultures and washed and then 10^6 cells in 300ul of medium were in subcutaneously in mammary fat pad of rats.

Experimental Design:

Total requirement of 9 Sprague Dawley female rats divided in to 4 group and each group containing 3 female rats.

Group-1: Positive Control group.

Group-2: Negative Test group containing 3 female rats treated with cell line 300ul cell suspension injected slowly every 5 min in mammary fat pad (subcutaneously) by TB syringe.

Group-3: Test group containing 3 female rats treated with formulation treated curcumin 25 mg/kg (p.o.) 0.5ml.

Group-4: Test group containing 3 female rats treated with free curcumin 25mg/kg (p.o.) 0.5 ml.

The experiment lasted for three weeks.

PROCEDURE-

ANTICANCER -

IN VITRO METHOD-

Tetrazolium salt assay (MTT Assay) (Debnath et. al. 2013)

Procedure:

Aliquote of 200ul cell suspension were plated in 96-well tissue culture plates (SPL life sciences co, korea) 1000 cells/well) and cells were treated with different concentration of generation 3 PAMAM dendrimer encapsulating curcumin (0.5-60uM)(kindly donated by Ardebil polymer research center).Also some wells were considered as appropriate control (culture medium, cell control, PAMAM dissolved in PBL). The same concentrations were used for free curcumin dissolved in DMSO, in addition to appropriate controls (culture medium, cell control, DMSO). For analysis of cell viability Trypan blue (Sigma Aldrich) was used. After 24, 48 and 72h exposure times, medium was replaced with fresh medium and let cells proliferate for two to three population- doubling time (PDTs) and then 50ul 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1mg/ml)was added to each well. The plates were incubated for 4h, allowing viable cells to reduce the yellow MTT to dark-blue formazan crystals, and then were dissolved in 200ul DMSO and 25ul Glycine Buffer (Sigma Aldrich). Finally absorbance of each individual wells was determined at 595nm using a micro plate reader STAT FAX 2100.

Cage side observation:

In this study, animal were observed individually after drug administration and special attention was given during the first 4hours and daily observed for three weeks. All observations were systematically recorded, with individual records being maintained for each animal. Cage side observation included coloration of skin and fur, eyes, color of toungs, autonomic effects such as salivation, urination, faecal matter, behavior, mortality and morbidity etc.

Body weight measurement:

Body weight of all groups of rats were recorded at weekly intervals at 1st, 7th,14th, 21th days through out of the study by using a sensitive Dolphin digital balance and showed changed in body weight compared with control group.

Food and water measurement:

The amount of food and water consumption ware measured daily from the quantity of food water supply and the amount remaining after 24 hour for three week of study period.

HEMATOLOGICAL STUDY:

Blood samples of control and treated group were collected from tail- vein of animals once before commencement of dosing and every 7th day for three weeks of observation period. Collected blood sample was kept in sterilized EDTA vials. Hematology analyses were performed on whole blood, to evaluate the following parameters: erythrocyte count (RBC), hemoglobin (HGB), differential leucocytes count (DLC), mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC), platelet count and leukocytes count(WBC).

HISTOPATHOLOGICAL STUDY:

At the end of the study, all organs were collected using standardized surgical procedures. The entire animals were euthanized and dissected through a central abdominal incision. The liver, kidney, lung, heart, spleen, testis and brain samples were collected and immediately fixed in 10% formalin (pH 7.4) in labeled sample 50ml tarson tubes. The tissues were dehydrated in graded concentration of xylene, embedded in molten paraffin wax and sectioned at 5u.the tissues section were fixed on grease free glass slides and stained with hematoxylin-eosin for microscopy at 10x, 45x and 90x. photomicrographs of some of the tissues were using a microscope fitted with camera unit and processed routinely in color photographs.

Absolute and relative organ weight:

At the end of the study, all organs were collected using standardized surgical procedures. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidney, brain, female reproductive organs were quickly removed, cleaned with saline, weighed and preserved in 10% formalin for histopathology.

IN VIVO METHODS:

Cell line MCF-7 Cell induced Mammary fad pad-

Procedure-

Female SD rats animal will be taken 180-200gm body weight .Divided in to four groups per groups 3 animals for cage. Group –1: positive control, Group-2: Negative control cell line 300ul cell suspension injected slowly every 5 min in mammary fat pad (subcutaneously) by TB syringe MCF-7 cells ($1*10^7$ml)17 B estradiol (i.p) mammary fat pad subcutaneously 300 ul. After 2 weeks palpable tumors develops further animals divided into groups normal untreated, control treated with saline (inject of physiological saline), test group CDP-4, test group for curcumin, stand i.p. heamatological till 4 weeks behavioural and pathological parameters was assayed. Drug were injected subcutaneously daily for 28 days. After 4 weeks animal were sacrified for tumor growth study.Group-3: Test group formulation treated curcumin 25mg/kg (p.o.) 1ml .Group-4: Test group treated with free curcumin 25mg/kg (p.o.) 0.5 ml.

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Blood samples of control and treated group were collected from tail- vein of animals once before commencement of dosing and every 7^{th} day for three weeks of observation period. Collected blood sample was kept in sterilized EDTA vials. Hematology analyses were performed on whole blood, to evaluate the following parameters: erythrocyte count (RBC), hemoglobin (HGB), differential leucocytes count (DLC), mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC), platelet count and leukocytes count(WBC).

HISTOPATHOLOGICAL STUDY:

At the end of the study, all organs were collected using standardized surgical procedures. The entire animals were euthanized and dissected through a central abdominal incision. The liver, kidney, lung, heart, spleen, testis and brain samples were collected and immediately fixed in 10% formalin (pH 7.4) in labeled sample 50ml tarson tubes. The tissues were dehydrated in graded concentration of xylene, embedded in molten paraffin wax and sectioned at 5u.the tissues section were fixed on grease free glass slides and stained with hematoxylin-eosin for microscopy at 10x, 45x and 90x. photomicrographs of some of the tissues were using a microscope fitted with camera unit and processed routinely in color photographs.

Absolute and relative organ weight:

At the end of the study, all organs were collected using standardized surgical procedures. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidney, brain, female reproductive organs were quickly removed, cleaned with saline, weighed and preserved in 10% formalin for histopathology.

ANTICANCER MODEL:

In vitro method-

Tetrazolium salt assay (MTT Assay) (Debnath et. al. 2013)

- It is perfirmed to determine the enzymatic properties
- The cell from a particular cell line when in logphase a trysinized
- It is counted in a homocytometer and adjusted multiwall plates (96 well plates)
- The cells are treated with a various concentration of drug for specified duration
- After MIT dye is added in each well and plates are incubated at 37 0 C for 4 hrs in a CO 2 incubator
- The plates are taken out the incubator and dark –blue colored formazan crystal arethoroughly dissolved in DMSO in room temperature.
- The plates are the read on a ELISA reader at 570 nm.

Sulphorhodomine B assay(Adams et. al., 2004)

- The sulphorhodamin B assay measures whole-culture protein content, which should be proportional to the cell number.

- Cell culture are stained with a protein staining dye, sulphorhodamin B
- SRB is a bright pink anionic dye that binds to basic amino acid of cell
- Unbound dye is then removed by washing with acetic acid.

IN VIVO METHOD-

DMBA –induced rat mammary gland carcinogenic(Jabara, A.G.,et al.)

- Female Sprague- dawley are use for this method
- Rats are given single intragastric injection of 12 mg/kg DMBA at 50 days of age
- This dose results in 80-100% incidence of total mammary tumors with in 120 days post carcinogen.
- This model can detect the agents/drugs inhibiting carcinogen activation
- DNBA produced capsulated tumors with high incidence.
- Drug efficacy is measured as percent reduction in adenoma incidence, percent increase in adenocarcinoma latency compared with that of carcinogen control.

DISCUSSION AND CONCLUSION:

A polyphenol called curcumin (diferuloylmethane) is obtained from the plant Curcuma longa, also known as turmeric. Research has shown that this polyphenol can both prevent and treat cancer during the past 50 years. The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of tumour cells, down-regulate transcription factors NF-κB, AP-1 and Egr-1; down-regulate the expression of COX2, LOX, NOS, MMP-9, uPA, TNF, chemokines, cell surface adhesion molecules and cyclin D1;down-regulate growth factor receptors (such as EGFR andHER2); and inhibit the activity of c-Jun N-terminal kinase, protein tyrosine kinases and protein serine/threonine kinases.

The insolubility of curcumin in water, which results in low absorption and hence poor bioavailability, is one disadvantage.

Being a well-defined and highly branching macromolecule, dendrimers are recognized for their potent multifunctionality and distinctive shape. terminal groups potent for interaction of oppositely charged component and produces surfactant like effects by forming a nanoparticles for drug delivery. In the context of drug delivery, the use of fatty acids as permeation enhancer limits the metabolism of drug in liver when administered orally and allowed for plasma binding in blood readily. The application f this study could be sustained for oral delivery and improvd absorption through gastrointestinal tract for overall enhancement in therapeutic efficacy.

These reports served as the foundation for the current study, which aims to overcome pharmacokinetic limitations by conjugating with dendrimer, comprehending the physicochemical properties of the curcumin/dendrimer/fatty acids complex system, and assessing its suitability for sustained drug release. This release is further permitted for oral drug delivery for screening as potent anticancer agents by using in vitro and in vivo model.

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