ANTITUMOUR ACTIVITY OF CURCUMIN IN ALLIUM CEPA INDUCED BY AGROBACTERIUM TUMEFACIENS

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Abstract

Cancer is a general term applied to malignant diseases characterized by rapid and uncontrolled abnormal cells formation which may mass together to form a growth or proliferate throughout the body, and it may progress until it causes death. (Amaro *et al*, 2008). Cancer is not just one disease but many diseases. The available experimental evidence suggests that it is worth testing curcumin as a cancer therapeutic agent. In this study, curcumin has been extracted from *Curcuma longa* through distillation with ethanol. Curcumin has been qualified using reverse phase HPLC. This study reveals that curcumin inhibit cancerous cell growth at a concentration of 1.5%, incubated for 2 weeks and induce apoptosis in *Agrobacterium* transformed *Allium cape* callus culture.

(Key words: Cancer, Curcumin, ***Agrobacterium tumefaciens)***

Introduction

Pharmaceutical Microbiology

Pharmaceutical microbiology is the part of industrial microbiology that is responsible for creating medications. All parenteral drugs, including many oral drugs, must go through rigorous microbiological testing in order to validate certain compounds by United States Pharmacopeia regulations. There is global recognition that pharmaceutical products must always be • Effective for the therapeutic purposes for which they are prescribed • Free from side effects that could make them unsafe to use • Free of chemical, physical or microbiological contaminants that may adversely affect their efficaciousness and safety. Turmeric holds a high place in Ayurvedic medicine as a "cleanser of the body" and today science is finding a growing list of diseased conditions which turmeric's active ingredient heals. Broad interest in curcumin's anti-inflammatory effects is increasing.

Researchers are examining curcumin as a possible imune system stimulator that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells; downregulate various proinflammatory cytokines and chemokines, and enhance antibody responses. A study of curcumin to prevent cataracts found, unexpectedly, that in rats low doses indeed did lower cataract rates but heavy doses raised the rate of cataracts (Molecular Vision 2003) Biochemists in China reported on January 2007 that curcumin “downregulates homeobox gene NKX3.1 in prostate cancer cell LNCaP” and could also dampen the androgen receptor's sensitivity to this gene. Independent studies have found that the Nkx3.1 homeobox gene has a key role in the prostate and may be implicated in startup of prostate cancer.

Several studies indicate that curcumin slows the development and growth of a number of types of cancer cells. In Japan 2003, researchers defined curcumin as a broad-spectrum anti-cancer agent. Its induction of “detoxifying enzymes,” the researchers say, indicates its "potential value ... as a protective agent against chemical carcinogenesis and other forms of electrophilic toxicity. The significance of these results can be implicated in relation to cancer chemo preventive effects of curcumin against the induction of tumors in various target organs" (Iqbal *et al*., 2003).

Pharmacologically, curcumin has been found to be safe. Human clinical trials indicated no dose-limiting toxicity when administered at doses up to 10 g/day. All of these studies suggest that curcumin has enormous potential in the prevention and therapy of cancer." (Aggarwal *et al*, 2003)Several breast tumor cell lines, "including hormone-dependent and independent and multidrug-resistant (MDR) lines," respond to antiproliferative effects of curcumin. Aggarwal *et al* examined cell lines "including the MDR-positive ones," and found they were all "highly sensitive to curcumin. The growth inhibitory effect of curcumin was time- and dose-dependent.... Overall the results suggest that curcumin is a potent antiproliferative agent for breast tumor cells and may have potential as an anticancer agent." (Aggarwal *et al*., 1997).

Cancer

Cancer is a general term applied to malignant diseases characterized by rapid and uncontrolled abnormal cells formation which may mass together to form a growth or proliferate throughout the body, and it may progress until it causes death. (Amaro *et al*, 2008).Cancer is not just one disease but many diseases. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start - for example, cancer that begins in the colon is called colon cancer; cancer that begins in basal cells of the skin is called basal cell carcinoma. Cancer types can be grouped into broader categories. The main categories of cancer include:

- **Carcinoma** - cancer that begins in the skin or in tissues that line or cover internal organs.
- **Sarcoma** - cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- **Leukemia** - cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
- **Lymphoma and myeloma** - cancers that begin in the cells of the immune system.
- **Central nervous system cancers** - cancers that begin in the tissues of the brain and spinal cord.

Chemoprevention

Chemoprevention is a promising anticancer approach with reduced secondary effects in comparison to classical chemo therapy. It was described as the use of natural or synthetic chemicals allowing suppression, retardation or inversion of carcinogenesis. Most chemo preventive agents known until today are plant extracts subdivided into two classes.

(1) Blocking agents, which inhibit the initiation step by preventing carcinogen activation and (ii) suppressing agents, which inhibit malignant cell proliferation during promotion and progression steps of carcinogenesis. Curcumin, one of the most studied chemo preventive agents, is natural compound extracted from curcuma longa (Annelyse Duvoix *et al.*, 2004

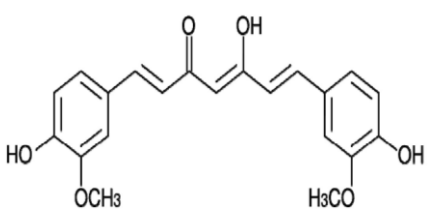
Curcuma longa

Turmeric (*Curcuma longa*) is extensively used as a spice, good preservative and coloring material in India, China and South East Asia (Ishita Chattopadhyay *et al*, 2004). Turmeric is a member of the ginger family (Zingiberaceae family). Turmeric contains three major phytochemical compounds (called curcuminoids), which give turmeric its bright yellow-orange colour. These curcuminoids have been the focus of numerous clinical studies investigating their long term safety, antioxidant properties, and anti-microbial and anti-

inflammatory activity. Curcuminoids include mainly curcumin (diferuloylmethane), dimethoxy curcumin, and bisdemethoxycurcumin curcumin. (Simay Cikirikei *et al.*,2008). Curcumin is the most important fraction which is responsible for the biological activities of turmeric.

1.5. Curcumin

Curcumin (diferuloyl methane), a polyphenolic compound (molecular formula C_{21} H_{20} O_6) isolated from the plant *Curcuma longa*, has been widely used as a spice and colouring agent (Arayjo and Leon, 2001; Goel *et al.*, 2008). Curcumin acts as a potent antioxidant, anti-inflammatory and antiproliferative therapeutic agent (Jagetia and Aggarwal, 2007). Commercially, curcumin contains approximately 77 %, diferuloyl methane, 17% demethoxy curcumin, and 6% bisdemethoxy curcumin (Goel *et al.*, 2008)



The Structure of Curcumin

Curcumin can act as a potent immunomodulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells (Gaurisankar and Das,2008; Jagetia and Aggarwal, 2007). Several documents have documented that curcumin can induce apoptosis in cancer cells from liver, colon, breast, stomach and duodenal tissue (Shi *et al*; 2006)

Pharmacological Action of Curcumin

Curcumin has been shown to protect the stomach from ulcerogenic effects of phenylbutazone. Turmeric powder has beneficial effect on the stomach. It increases mucin secretion in rabbits and may thus act as gastroprotectant against irritants. (Ishita Chattopadhyay *et al.*,2004). Curcumin is reported to have antibacterials, antiamoebic and anti HIV activities. Curcumin also shows antioxidant activity. It also shows antitumour and anticarcinogenic activities.

Anti-Carcinogenic Activities Of Curcumin

Numerous research teams provided evidence that curcumin contributes to the inhibition of tumour formation and promotion as Cancer initiation, promotion or progression of tumours is decreased or blocked by this compound.Anticancer effect of curcumin seems to be potentialized in the presence of Oestrogen in breart Cancer cells and it inhibits genes which are under the influence of the oestrogen receptor .Curcumin also displays an inhibiting effect on human telomerase reverse transcriptase expression reducing telomerase activity in MCF-7 cells. Moreover, it allows sensitizing ovarian cancer cells to eiplabin, enhancing chemotherapeutic treatment.

Contributionof Curcumin to the Induction Of Apoptotic Mechanisms:-

The ability of curcumin to induce apoptosis in cancer cells without cytotoxic effects on healthy cells contributes to the understanding of the anti-cancer potential of curcumin. This spice is described to efficiently induce apoptosis in various cell lines including HL-60, MCF -7 and HeLa. Curcumin also leads to apoptosis in Scleroderma lung fibroblasts. This effect

seems to be due to the weak level of protein kinase in SLF, generating low levels of glutathione S-transferare (GST) P1-1.

Allium cepa

Onion is a term used for many plants in the genus Allium. It is grown underground by the plant as a vertical shoot that is used for food storage. They contain chemical compounds believed to have anti-inflammatory, anticholesterol, and anticancer and antioxidant properties such as querectin. The medicinal use of onion is due to 3- Mercapto-2-methylpentan-1-0l compound present in it.

Agrobacterium tumefaciens

Agrobacterium is a genus of Gram negative bacteria that uses horizontal gene transfer to cause tumors in plants. *A.tumefaciens* is the most commonly studied species in this genus. *Agrobacterium* is well known for its ability to transfer DNA between itself and plants, and for this reason it has become an important tool for plant improvement by genetic engineering. The *Agrobacterium* genus is quite heterogenous. Recent taxonomic studies have reclassified all of the *Agrobactecium* species into new genera, such as *Ruegeria, Pseudorhodobacter* and *Stappia,* but most species have been reclassified as *Rhizobium* species.

Agrobacterium tumefaciens causes crown gall disease in plants. The crown gall formation is due to the conjugative transfer of T-DNA from Ti-plasmid in the bacteria. The plasmid T-DNA is integrated Sem-randomly into the genome of the host cell (Francis and Spiker,2005), and the virulence (Vir) genes on the T-DNA are expressed, causing the formation of a gall. The T-DNA carries genes for the biosynthetic enzymes for the production of unusual amino acids, typically octopian or nopaline. It also carries genes for the biosynthesis of the plant hormones, auxin and cytokines. By altering the hormone balance in the plant cell, the division of those cells cannot be controlled by the plant, and tumors form. The ratio of auxin to cytokinin produced y the tumor genes determines the morphology of the tumor. (root like, disorganized or shoot –like).

1.11. Crown Gall

Crown gall tumors can be maintained as undifferentiated masses of tumorcells in tissue cultures without the infecting bacteria and added hormones required for cell culture of normal tissues. Upon grafting onto an intact shoot, some develop meristematic zones of cells that initiate the formation of small, distorted shoots, or teratomas(Lippincott& Lippincott 1975) . In other cases, the shoots formed are similar to those of normal plants but retain the capacity of tumor growth. They can even appear to have completely recovered from the transformed state. Teratoma structure of shoots, however, has a short life span; the stems die, and the tumors decay.

Materials and Methods

Sample Collection:

Rhizomes of *Curcuma longa* were obtained from the fields of Kerala and TamilNadu.

Extraction of Curcumin

Distillation

The process of extraction requires the raw material to be ground into powder, and washed with a suitable solvent that selectively extracts colouring matter. Distillation apparatus was set and using different solvents isopropanol,ethyl acetate,acetone and finally distillation was done using methanol. This process of distillation yields an oleoresin with colouring matter

content in the region of 25-35 percent along with volatile oils and other resinous extractives. The oleoresin so obtained is subjected to further washes using selective solvents that can extract the curcumin pigment from the oleoresin. This process yields a powdered, purified food colour, known as curcumin powder, with over 90 percent colouring matter content and very little volatile oil and other dry matter of natural origin.

Curcumin Quantification Using HPLC

Determination of Analysis Condition

a**. Mobile phase preparation**

1. An amount of 20 mL glacial acetic acid measured then put into 1 L beaker glass; 980 mL aquabidestilata was then added. The mixture was stirred using magnetic stirrer for 10 minutes to obtain homogeneous solution. The solution was then filtered by pump filter; the filtrate was placed in Erlenmeyer flask and degassed. The solution was then placed into mobile phase bottle and labeled FG-Ac 2%.

2. An amount of 1 L acetonitrile was placed in beaker glass. The solution was filtered by pump filter then placed in mobile phase bottle and labeled FG-ACN.

b. **Instrument Optimization**

HPLC column was cleaned up by elution, filtration, and degas ion. Elution runs for 1 hour, then the column was washed using acetonitrile for 1 hour. After the washing step, the column was conditioned by eluting mobile phase 2% acetic acid and acetonitrile (45:55) for 30 minutes and at the same time it was run for baseline.

c**. Working solution preparation**

For standard solution 100 ppm, 50 mg curcuminoid standard was weighed accurately, diluted in 50 mL volumetric flask until the limit mark with acetonitrile, then 2.5 mL was taken and diluted into 25 mL volumetric flask with the same solvent. Procedures for other standard concentrations were done similarly.

d. **Linearity and Range Study**

Six concentrations of curcuminoid standard solution were made: 5, 10, 20, 40, 80, and100 ppm. Each solution was filtered by syringe filter 0.45 μm. The solutions were injected into injector once for each time and the area under curve was recorded and measured for the r2 and r values.

e. **Presicion Study**

Ten solutions of 20 ppm curcuminoid were made for standard solutions. Each solution was filtered by syringe filter 0.45 μm. The solutions were injected into injector once for each, and then the area under curve was recorded and measured for the coefficient variation values (CV1).

f**. Accuracy Study**

Three concentrations of curcuminoid standard solution were prepared for 20, 40, and 80 ppm. Each solution was filtered by syringe filter 0.45 μm. The solutions were injected into injector three times for each solution and then area under curve was recorded and measured for the average recovery values.

g**. Limit of Detection and Limit of Quantification Study**

Six concentrations of curcuminoid standard solution were prepared: 5, 10, 20, 40, 80, and 100ppm. Each solution was filtered by syringe filter 0.45 μm. The solutions were injected

once for each time and then the area under curve was recorded and measured for the standard deviations, limit of deviation (LOD), and limit of quantitation (LOQ) values

h. Standard curve estimation

Six concentrations of curcuminoid standard solution were prepared: 5, 10, 20, 40, 80, and 100ppm. The solutions were injected into injector once for each and the area under curve was recorded and measured for the correlation coefficient in linear regression equation (Y = ax + b).

Quantitative Analysis

a. **Sample solution preparation**

1. Dosage form sample. Two samples of tablet were weighed and powdered to homogeneous. The powder sample was weighed for 1 dosage and diluted into 25 mL volumetric flask; 5 Ml.

The solution was taken and diluted with the same solvent into 10 mL in a volumetric flask. The solution was filtered with 0.45 μm Whatman paper, and placed into HPLC vials. For capsule dosage form, similar steps were carried out as for tablet, with adjusted dilution for curcuminoid concentration in sample.

2. Curcuminoid raw material sample. Fifty mg curcuminoid sample was weighed accurately, diluted in 50 mL volumetric flask with acetonitrile, and then 2.5 mL of the solution was taken and diluted into 25 mL volumetric measure flask with the same solvent. The solution was filtered with 0.45 μm Whatman paper, and placed into HPLC vials.

b**. Concentration measurement**

Eighty ppm solution from the stock solution of four samples was injected into injector twice for each sample. The area under curves were recorded and plotted into standard curve linear regression equation.

4.4. Callus Culture

The required parts of the explants from *Allium cepa* were cut and the dead and unwanted parts were removed after wiping with alcohol. The pieces were submerged in tap water containing a few drops of surfactant and was shaken continuously for 20 minutes. This was followed by washes with 1% emicin solution and 1% Bavistin solution for 20 minutes. Thee explants were taken to the LAF chamber after a wash with distilled water. The explants were rinsed with 70% ethanol for less than a minute. They were transferred to a solution of 0.12% Mercuric chloride containing 1 to 2 drops of 5% teepol and stirred continuously for 15 minutes. An immediate wash with sterile distilled water was given and followed by 3-5 washes with a gap of 5-10 minutes. The dead tissue was trimmed off and the viable part was inoculated in to sterile MS media using sterile forceps and scalpels. The cotton plugs were replaced and the flasks were incubated in the growth room in dark under controlled condition of humidity and room temperature. The culture vessels were observed weekly and the changes were noted.

MURASHIGE-SKOOG MEDIA

CONSTITUENTS	**MG/L**
Macronutrients	
NH_4NO_3	1650
KNO_3	1900
$CaCl_2.2H_2O$	440

$MgSO_4.7H_2O$	370
KH_2PO_4	
Micronutrients	
KI	0.83
H_3BO_3	6.2
$MnSO_4 4H_2O$	22.3
$ZnSO_4 7H_2O$	8.6
$Na_2MoO_4 2H_2O$	0.25
$CuSO_4 5H_2O$	0.025
$CaCl_2 6H_2O$	0.025
EDTA	43.00
Vitamins and Hormones	
Inositol	100.0
Nicotinic acid	0.5
Pyridoxine-HCl	0.5
Thiamine-HCl	0.1
IAA	1-30
Kinetin	0.04-10
Carbon Source	
Sucrose	30000.0
p^H	5.7

Regeneration of Callus In Suspension Media

Using a burner the neck of the flask containing the liquid media was sterilized. The callus pieces were taken in sterile petri dish and using a sterile forcep and it was broken into pieces. Under sterile condition the small pieces were transferred in to sterile liquid media. After pluging with cotton, the flasks were incubated for 1-2 weeks on a shaker at 125rpm.The samples of the suspension cultures were taken using a sterile large-bone pipette to observe under microscope. The culture was taken on a haemocytometer, and viewed under microscope(45X) after the addition of a drop of methylene blue. The proportion of dividing cells during the development of suspension culture was estimated.

Isolation of *Agrobacterium tumefaciens*

The diseased nodule was obtained from *Pongamia pinnata* . The soil was removed from the affected tissue and rinsed well in distilled water. The tissues were surface disinfected within 10% household bleach (1 part bleach:9 parts water)for 1-3minutes. The affected piece of tissue about 5 mm diameter was aseptically removed and placed into a tube each of 1A and 2E broth and sonicated for 60 minutes. The tubes were incubated for 1-3 days at 27º C. The tubes were vortexed. Using a sterilized and cooled bacteriological loop culture was streaked from each tube onto its corresponding agar medium; e.g. streak from 1A broth onto 1A agar plates. And incubated for 3 days at 27ºC.*Agrobacterium* were observed for domed, mucoid colonies. The colonies usually have transparent margins and may have some purple, red (1A) or green (2E)

color in the centers. The selected colonies were transferred to fresh media for purification. A part of colony was picked with a sterile loop and inoculated into a tube of 5 ml sterile deionized water or saline .The tube was vortexed and streaked onto MGY media and incubated for 3 days at 27º C.The same procedure was repeated for two or more times or until colonies look uniform in size, shape, and morphology. The colonies on MGY that fluoresence under a black light (366 nm) were not considered since these will be *Pseudomonads*. The *Agrobacterium* colonies on MGY are white, mucoid, domed, and opaque. The 24 hr-old cultures were Gram stained to check for purity. The oxidase test was performed. All putative isolates are then put through Biolog to confirm identity and to check for pathogenicity, the isolate was inoculated onto *Bryophllum daigremontianum.*

	MGY
Deionized water	1000 ml
Mannitol	10 g
L-glutamic acid (sodium salt)	2 g
KH_2PO_4	0.5 g
NaCl	0.2 g
$MgSO_4$ $7H_20$	0.2 g
Adjust pH to 7.0 then add,	
Yeast extract	1.0 g
Agar	15 g

Dissolve the agar before autoclaving for 20 minutes at 15 psi and 121 C

Primer Designing

The nucleotide sequence of the vir A gene in the isolated Agrobacterium tumefacians species was accessed from NCBI data base. Using primer3 software the primer sequence required for the amplification of vir A gene in the isolated Agrobacterium tumefacians was designed. The primer sequence used in this work was

Forward primer 5’ CGCTTCCACACTGCTGTTC 3’ and

Reverse primer 5’GATGCTCGGGTTGGAATAGA 3’

Detectionof Pathogenic *Agrobacterium tumefacians* Using PCR

COMPONENTS OF REACTION MIXTURE

- Primers,forward,reverse (sigma)
- dNTP mix (fermentas)
- Taq Buffer (fermentas)
- Magnesium chloride (fermentas)
- Taq DNA polymerase (fermentas)
- Template D

All the solutions were gently vortexed and briefly centrifuged after thawing. The sample was added to a thin walled PCR tube, on ice and was gently vortexed and briefly centrifuged to collect all drops from the walls of the tube. The samples were placed on LONGENE MG25+ thermal cycler equipped with a heated lid and the reactions were started. Reactions were carried out in a final volume of 25µl mixture consisting of:1µl of crude bacterial cell lysate,1µl of sigma primers.

Forward primer 5’ CGCTTCCACACTGCTGTTC 3’ and

Reverse primer 5’GATGCTCGGGTTGGAATAGA 3’

2 Units of Fermentas Taq DNA polymerase, 0.5 mM of each dNTP, and 5 mM MgCl2 in a 1x PCR buffer (provided with Fermentas Taq)reactions were conducted simultaneously on all isolates in a PCR tubes in a LONGENE MG25+ thermal cycler with the following protocol: denaturation for 3 min at 94°C, followed by 30cycles of 30 sec at 94°C, 1 min at 32°C, 2 min at 68°C with a final extension step of 10 min at 72°C for 30 cycle

Electrophoretic Separation Of PCR Product

The PCR product obtained after amplification was run on 1.5% agarose gel .To 50ml of TE buffer, 1g of agarose was dissolved by heating and the agarose solution was cooled to 60 °C and 2ml of ethidium bromide was added. The sides of the gel casting plate were covered and then combs were placed. The agarose solution was poured on the gel casting tray and allowed to solidify.

The samples were mixed with the gel loading dye (2ml) and was loaded into the well. The electrophoretic unit was run at 70-75 volt for one hour. The DNA present in the samples was viewed by placing agarose gel in TU1002 UV transilluminator at 312nm.

Culturing Of Pathogenic Strains To Subculture Callus Suspension

The calli was gently passed through 2000µm mesh polypropylene filters. Placed the sieved calli on petridish and dried for 10-15 minutes under laminar air flow. 2g of calli is transferred to a petridish and added 2ml of *Agrobacterium* suspension in exponential growth (OD_{600}= 1).After 10minutes washed the infected calli 2 times for 1 minute with distilled water and dried in a sterile filter paper or sterile paper towel. The infected calli was transferred to a semisolid (0.2g/L agar) plant regeneration media for cocultured for 24hr (in the dark at 25^oC).The infected media was kept at dark for 12 days to regenerate cancerous callus for further studies .

Plating The Tumorous Callus In Ms Medium Withdifferent Curcumin Concentrations (0.5%,1%,1.5%)

MS medium containing (0.5%,1%,1.5%)curcumin were prepared. After completing the co culture period, the calli was transferred into the prepared MS medium. The cultures were tncubated at 25^0C, 16hours light photo period for 4 weeks. Proper control plates were also maintained for comparing the results .

Results

Extraction of curcumin

Powdered curcumin was obtained after distillation. (Plate1)

HPLC

With this analytical method, curcumin concentration in samples has been determined for samples A, B, C, and D (from the concentrations written in the label) as 105.86,87.12, 10.71, and 130.35%, respectively.

Callus Culture

The young callus culture of onion plant was obtained after incubation with MSA and followed by callus suspension regeneration media (Plate 2)

Isolation of*Agrobacterium tumefacians*

A both virulent and non virulent strain of *Agrobacterium tumefacians* were isolated from crown gall and was examined for further cancer development in the regenerated callus (Plate 3).

Primer Designing

For identifying the virulent strains of *Agrobacterium* PCR Technique was used for performing serial PCR reactions primers were designed using DNA softwares and was used for further reactions. **Detection of Pathogenic *Agrobacterium tumefacians* Using PCR**

Using the designed primers PCR was carried out by isolating the crude bacterial lysate DNA as template.

Electrophoretic Separation Of PCR Product

The obtained product was electrophoresed and was viewed under UV trans illuminator .pink bands of Vir a gene were obtained with a base pair size of 304 bp using standard markers .(Plate 4)

Regeneration of Callus with Virulent Strain of *A.tumefaciens*

The regenerated callus was incubated with pathogenic *Agrobacterium tumefaciens* after 12 days of incubation a cancerous growth near the callus was seen.(Plate 5)

Plating theTumorous Callus In MS Medium With Different concentrations (0.5%, 1%, 1.5%) of Curcumin

After plating with different concentrations of Curcumin it is found that

0.5%curcumin containing plate showed little growth after incubation of cancerous callus for 2 weeks (Plate 8).

1%curcumin containing plate showed neither significant growth nor destruction after incubation of cancerous callus for 2 weeks (Plate 9).

1.5%curcumin containing plate showed complete destruction of the culture after incubation of cancerous callus for 2 weeks (Plate 10).

Hence it is concluded that the 1% Curcumin (0.01gin 1 ml) has significant activity showed under normal conditions of a cancerous tissue.

Discussion

The focus of this study was to assess the anticarcinogenic activity of curcumin. Curcumin is extracted from *Curcuma longa* which belongs to the family Zingiberaceae. Curcumin is found to exhibit anti-inflammatory, anti-carcinogenic and anti-oxidant, anti-microbial and antiviral properties. In this study, *Agrobacterium tumefacien*s has induced tumors in *Allium cepa* and the infected cells have been treated with different concentrations of curcumin. It is found that 1.5%conceration of curcumin has inhibited the tumor like growth. Curcumin is the most important fraction which is responsible for the biological activities of turmeric.Curcumin has been shown to down regulate the expression of various pro inflammatory cytokines including TNF-α and interleukin –I through inactivation of the transcription factor NF-KB. Thus TNF-α and I α – Iβ could be identified as the therapeutic targets in cancer therapy. Current researches suggest that curcumin acts as a potent scavenger of reactive oxygen species and leads in apoptosis in several cancer cell lines. Nitric oxide(NO) and its derivatives play a major role in tumour promotion.Curcumin inhibits Inos and COX-

2production 69 by suppression of NFKB activation34 .Curcumin also increases NO production in NK cells after prolonged treatment,culminating in a strong tumouricidal effects .Curcumin also induces apoptosis in AK-5 tumour cells through upregulation 103 of capase-3 .Reports also exist indicating that curcumin blocks dexamethasoneinduced apoptosis of rat thymocytes.Recently, injurkat cells,curcumin has been shown to prevent glutathione depletion, thus protecting cells from capase-3 activation and oligonucleosomal DNA fragmentation. Curcumin also inhibits proliferation of rat thymocytes104.These strongly imply that cell growth and cell death share a common pathway at some point and that curcumin affects a common step,presumably involving modulation of AP-1 transcription factor.

Curcumin exerts both pro-and and antimutagenic effects .At 100 and 200 mg/kg body wt doses, curcumin has been shown to reduce the number of aberrant cells in Wistar rats. Turmeric also prevents mutation in urethane (a powerful mutagen)models. Contradictory reports also exist. Curcumin and turmeric enhance g-radiation-induced chromosome aberration in Chinese hamster ovary. Curcumin has also been shown to be non-protective against hexavalent chromium-induced DNA strand break .In fact, the total effect of chromium and curcumin is additive in causing DNA breaks in human lymphocytes and gastric mucosal cells.

Azuine *et al* ,1992; described curcumin as an inhibitor of tumour formation and promotion induced by benz(a)pyren,7, 12-dimethylbenz(a) anthracen or phorbol esters, while Ikezaki et.al 2001.demonstrated that curcumin inhibits cancer developments in rat stomach initiated by N-methyl-N –nitro-N-nitrosoguanidine (MNNG).In the sameway,bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione,abisdemethoxycurcuminanalog(BDMC-A),blocks the formation of colon adenocarcinoma in rats. Although curcumin does not decrease the copper-induced liver or kidney tumor incidence in Long-Evans Cinnamon(LEC)rats, an inbred mutant strain which accumulates copper due to an aberrant copper-transporting ATPase gene it reduces overall cancer formation as well as formation of metastasis.

The ability of curcumin to induce apoptosis in cancer cells without cytotoxic effects on healthy cells contributes to the understanding of the anti-cancer potential of curcumin .This spice is described to efficiently induce apoptosis in various cell lines including HL-60,k562,MCF-7 and Hela. Curcumin also leads to apoptosis in scleroderma lung fibroblasts(SLF)without affecting normal lung fibroblasts(NLF). This effects seems to be due to the weak level of protein kinase (PK) C in SLF, generating low levels of glutathione S-transferase(GST)P1-1.

Woo *etal 2003*; suggested that the induction of Caki (human kidney carcinoma cells)programmed cell death is activated by AKt dephosphorylation ,Bcl-2,Bcl-XL and inhibitor of apoptosis(IAP)protein inhibition ,as well as cytochrome C release and caspase-3 activation .These findings confirm results by Bush *et.al* 2001.,Anto *et.al* 2002 .and Pan *et al*2001.studying caspase 3 activation in melanoma and HL-60 cells. Bush *et al*2001.described that curcumin induces caspases 8 and 9,although p53 remains unchanged. Neverthless ,the death receptor pathway is activated through Fans in a Fas-Ligand indepent way. Anto *et al*. confirmed the role of Bcl-2 and Bcl-XL inhibition by preventing curcumin-induced apoptosis after over expressing these two key proteins. The present finding illustrates that suppression of tumor proliferation even in low doses of curcumin is due to the inhibitory effect of the curcumin by inhibition of various cytokines leads to apoptosis and involved in tumor proliferation or inhibiting RNA transcription.

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