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Cell culture study on the cytotoxic effects of "Cureit"- a novel bio available curcumin-anti cancer effects

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ABSTRACT

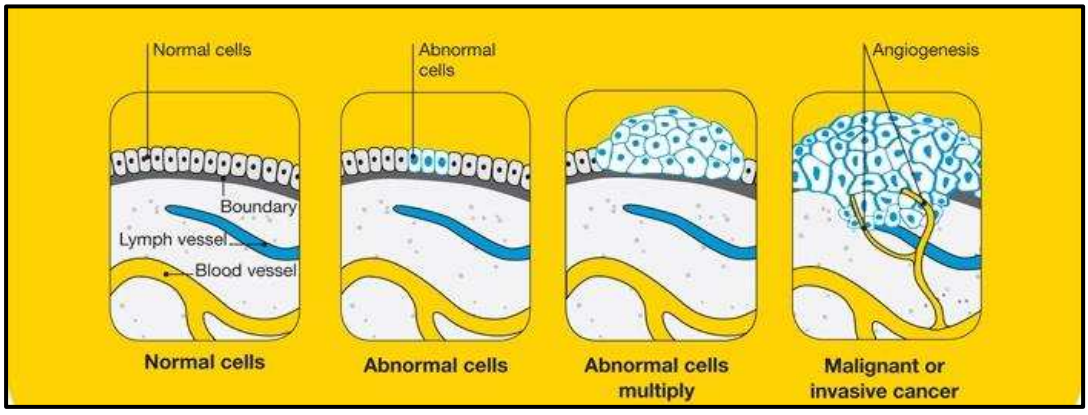
Cancer is a hyper proliferative disorder that metastasize into the vital organs in the body through invasion followed by angiogenesis and distant metastasis. Curcumin suppresses the proliferation of a wide variety of tumor cells, including breast carcinoma, colon carcinoma, renal cell carcinoma, hepatocellular carcinoma etc. The poor bio availability is the main drawback of the regular curcumin, which was addressed by Aurea biolabs and made a unique bio available formulation known as "cureit". The cytotoxic effect of cureit was established by a spectrophotometrical study using MTT on the effects of cureit on cell proliferation. It is inferred that the test sample – "cureit" could serve as an anti cancer medication.

Key words: Curcumin, tumor cells, cytotoxic effects, cancer

INTRODUCTION

Cancer is a hyperproliferative disorder that metastasize into the vital organs in the body through invasion followed by angiogenesis and distant metastasis. Within the last 25 years, much has been learned about the biochemical pathway that ultimately leads to cancer[1-3]. During the last decade, there has been extensive investigation of how Curcumin affects this overall process of tumorigenesis[4].

Figure1: Cancer in the body



Anti Proliferative Effects of Curcumin

Curcumin suppresses the proliferation of a wide variety of tumor cells, including breast carcinoma, colon carcinoma, renal cell carcinoma, hepatocellular carcinoma, T cell leukemia, B cell lymphoma, acute myelogenous leukemia, basal cell carcinoma, melanoma and prostate carcinoma. Additionally curcumin suppresses

the proliferation of certain normal cells such as hepatocytes, epithelial cells, human vascular endothelial cells (HVEC), human vascular smooth muscle cells (HVSMC), osteoclasts, peripheral bood mononuclear cells (PBMC) and T lymphocytes. Curcumin also inhibits the cell proliferation induced by growth factors[5-7].

Curcumin inhibits farnesyl protein transferase (FPTase):

Ras proteins must be isoprenylated at a conserved cysteine residue near the carboxyl terminus (Cys- 186 in mammalian Ras p21 proteins) in order to extend their biological activity. Previous studies indicate an intermediate in the mevalonate pathway, most likely farnesyl pyrophosphate, is the donor of this isoprenyl group, and that using inhibitors of the mevalonate pathway could block the transforming properties of ras oncogene. Chen *et al.* examined the effects of curcumin on farnesyl protein transferase (FPTase). They found that partially purified farnesyl protein transferase (FPTase) capable of catalyzing the farnesylation of unprocessed Rasp21 proteins *in vitro* was inhibited by curcumin and its derivatives [6]. This is another potential mechanism by which curcumin could suppress cellular growth.

Suppression of NF-κB activation by Curcumin

Members of the NF-κB transcription factor family play a central role in various responses leading to host defense, activating a rapid progression of gene expression. These transcription factors are dimeric complexes composed of different members of the Rel/NF-κB family of polypeptides. This family is distinguished by the presence of a Rel homology domain of about 300 amino acids that displays a 35% to 61 % identity between various family members[8-11]. Although NF-κB is a ubiquitous transcription factor, it plays its critical role in the cells of the immune system, where it controls the expression of various cytokines and the major histocompatibility complex genes. The inappropriate regulation of NF-κB and its dependent genes have been associated with various pathological conditions including toxic/septic shock, graft *vs* host reaction, acute inflammatory conditions, acute phase response, viral replication, radiation damage, atherosclerosis, and cancer.

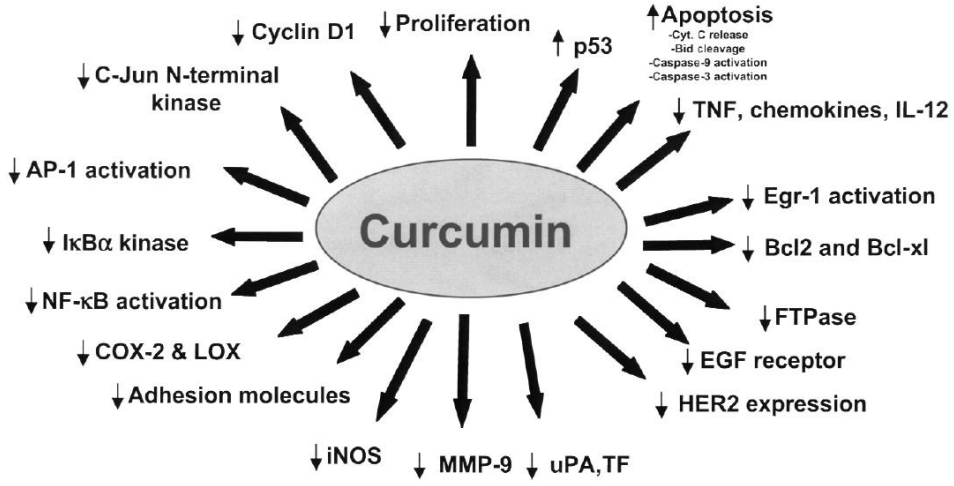


Figure 2: Molecular Targets of Curcumin

Bio Availability of Curcumin

It was known from the literatures that, the potential health benefits of curcumin are limited by its poor solubility, low absorption from the gut, and rapid metabolism. There are many bio available curcumin formulations available in the market, employing various additives to improve the bio availability. Aurea biolabs (A plantlipids company) developed a novel bio available curcumin formulation, completely in turmeric matrix, and its cytotoxic potential against cancerous cells was studied.

MTT Cell Proliferation Assay

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The action of dehydrogenase enzymes generate the reducing equivalents such as NADH and NADPH in the metabolically active cells, which causes the reduction of the yellow tetrazolium MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). The resulting intracellular purple formazan can be solubilized and quantified by means of spectrophotometer. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT

Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

The measurement of cell proliferation is based on the ability of the mitochondrial succinate-terazolium reductase system to convert 3-(4,5- dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) to a blue colored formazan. The test denotes the survival cells after toxic exposure.

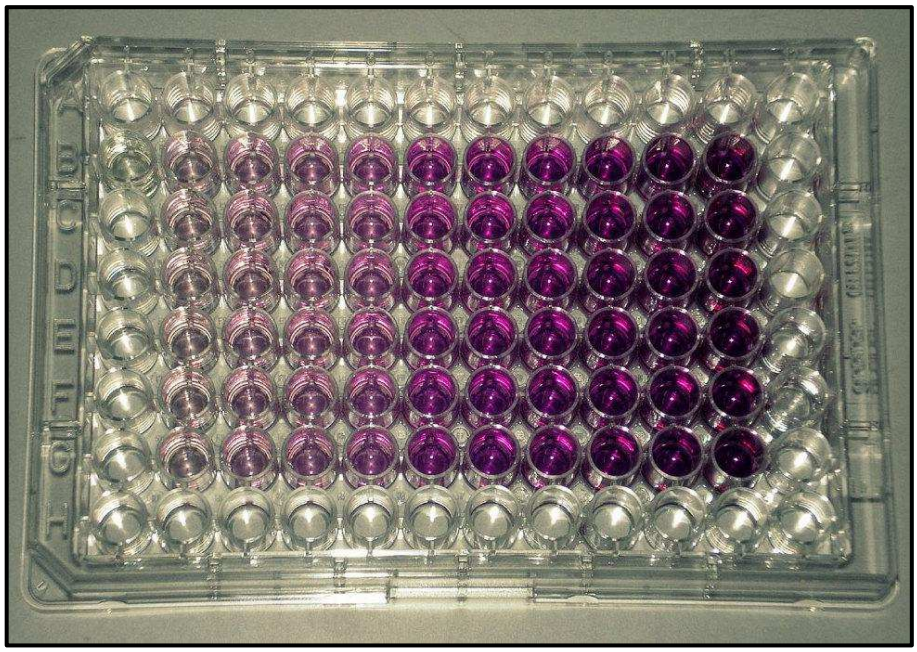


Figure 3: MTT Assay

Reagents and Materials Used

- Test Compound – "cureit"- Bio available curcumin
- MCF-7 (Breast Cancer Cell Line)
- LnCAP (Prostrate Cancer Cell Line)
- HEK 293 T (Normal human embryonic kidney cells)
- Minimal Essential Medium (MEM)
- RPMI (Rosewell Park Memorial Institute) medium
- DMEM (Dulbecco's modified Eagle's medium)
- FBS (Fetal Bovine Serum)
- MTT
- DMSO (Dimethyl sulfoxide)

Instruments Used

- Spectrophotometer
- Incubator

Methodology

MCF-7 (Breast Cancer Cell Line) was cultured in 5ml of Minimum Essential Medium (MEM) supplemented with fetal bovine serum(10%), L-glutamine(3%), penicillin (100IU/ml), streptomycin (100 µg/ml), amphotericin B (20 µg/ml), and phenol red. The pH of the medium was adjusted to 7.2-7.4 with 7.5% sodium bicarbonate and the flasks were incubated at 37°C in a humidified incubator (5% CO_2/95% O_2). LnCAP (Prostrate Cancer Cell Line) was cultured in RPMI (Roswell Park Memorial Institute) medium 1640 at 37°C in a humidified incubator (5% CO_2/95% O_2). HEK 293 (Normal human embryonic kidney cells) was cultured in DMEM (Dulbecco's Modified Essential Medium) at 37°C in a humidified incubator (5% CO_2/95% O_2). The cytotoxic effect of the test compound was studied on this non-cancerous cell line also. All three cell cultures were treated with the test compound – "cureit". 48 hours post treatment, the cells were treated with 100% MTT in media for 4 hours at 37°C in a humidified incubator (5% CO_2/95% O_2). The media was aspirated and the adherent cells

were dissolved in DMSO. This was centrifuged at 5000RPM for 15 minutes to remove debris. Then the absorbance was measured spectrophotometrically at 570nm.

RESULTS AND DISCUSSION

The following is the result of the assay performed.

Table 1: Cytotoxic Effect of Curcumin in MCF-7 cells

Sample	OD at 570nm					
Test sample (µg/ml)	Batch I	Batch II	Batch III	Avg	% Inhibition	%SD
Control	0.5891	0.6512	0.6222	0.6208		
10	0.6689	0.5912	0.6433	0.6345	-2.20	6.24
20	0.4865	0.5012	0.4621	0.4833	22.15	4.09
40	0.3561	0.36941	0.3625	0.3627	41.58	1.8
80	0.2564	0.3251	0.3625	0.3147	49.31	17
150	0.2213	0.2512	0.2021	0.2249	63.78	11

Table 2: Cytotoxic Effect of Curcumin in LnCAP cells

Sample	OD at 570nm					
	Batch I	Batch II	Batch III	Avg	% Inhibition	% SD
Control	0.7821	0.6891	0.5822	0.6845		
10	0.5676	0.6621	0.7012	0.6436	-3.68	10.6
20	0.5541	0.751	0.5421	0.6157	0.82	19.05
40	0.4568	0.4987	0.4751	0.4769	23.19	4.35
80	0.3211	0.3395	0.35005	0.3369	45.73	4.4
150	0.4156	0.3564	0.32	0.3640	41.37	13.26

Table 3: Cytotoxic Effect of Curcumin on HEK293 T cells

Sample	OD at 570nm					
	Batch I	Batch II	Batch III	Avg	% Inhibition	% SD
Control	0.6453	0.5643	0.5666	0.5921		7.8
10	0.7012	0.6972	0.6654	0.6879	-10.81	2.9
20	0.6754	0.7825	0.5671	0.6750	-8.73	16
40	0.6855	0.5769	0.5563	0.6062	2.35	11.5
80	0.6321	0.5911	0.5612	0.5948	4.19	6.0
150	0.3218	0.3092	0.5643	0.3984	35.82	36.1

The effect of the test compound – Curcumin was studied on the growth kinetics of MCF-7, LnCAP and HEK 293 T cells using MTT Assay. Dose response for the test compound- curcumin was studied in the cell lines in concentrations ranging from 10-150 µg/ml. MCF-7 cells seem to be more susceptible to the test sample induced cytotoxicity compared to LnCAP cells. It is inferred that the test sample – "cureit" could serve as an anti cancer medication.

CONCLUSION

The anti cancer properties of curcumin are already known in the scientific world. The poor bio availability is the main drawback of the regular curcumin, which was addressed by Aurea biolabs and made a unique bio available formulation known as "cureit". The cytotoxic effect of cureit was established and it is inferred that the test sample – "cureit" could serve as an anti cancer medication.

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