African Journal of Biotechnology Vol. 10(83), pp. 19481-19488, 21 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1228
ISSN 1684–5315 © 2011 Academic Journals

*Full Length Research Paper*

# β-Cyclodextrin-curcumin complex inhibit telomerase gene expression in T47-D breast cancer cell line

**Fatemeh Kazemi[1,3], Nosratollah Zaraghami[1]*, Sedighe Fekri aval[1] and Amir Monfaredan[2]**

[1]Drug Applied Research Center, Tabriz University of Medical Sciences, Faculty of Medicine, Department of Medical Biotechnology, Tabriz, Iran.
[2]Tabriz University of Medical Sciences, Faculty of Medicine, Department of Immunology, Division of hematology, Tabriz, Iran.
[3]Pasteur Institute of Iran, Tehran, Iran.

Accepted 5 October, 2011

**Today, attempt to the preparation of stable drug with high drug delivery efficiency is inevitable. Curcumin (diferuloylmethane), with hydrophobic structure obtained from the herb of *Curcuma longa*, have various applications in cancer therapy. But, its low water solubility and bioavailability is possible for poor drug delivery of curcumin. In this study, we prepared β-cyclodextrin-curcumin complex to determine the inhibitory effect of this drug on telomerase gene expression. Curcumin was encapsulated into cyclodextrin and the rate of curcumin loading was estimated. Cytotoxic effects of β-cyclodextrin curcumin were investigated by colorimetric cell viability (MTT) assay. Then inhibition of telomerase gene expression was determined by real-time polymerase chain reaction (PCR). MTT assay demonstrated that β-cyclodextrin have no cytotoxic effect on its own. Also, it showed dose-dependency and time-dependency for β-cyclodextrin –curcumin on T47D cell line. Expression of telomerase gene in cells effectively was reduced as the concentration of β-cyclodextrin –curcumin complex was increased. The results show that β-cyclodextrin -curcumin complex have cytotoxic effect on T47D cell line through down regulation of telomerase expression and induction apoptosis by enhancing curcumin uptake by cells. So, β-cyclodextrin could be good carrier for these kinds of hydrophobic agents.**

**Key words:** Anti cancer drug, target therapy, telomerase, breast cancer, drug delivery

## INTRODUCTION

Breast cancer is the second cause of cancer death in women (Tian et al., 2010). Telomerase, ribonucleoprotein enzyme maintained telomere length and inhibit cellular aging. In about 85 -90% of human cancer cells, including breast cancer, telomerase is overexpressed (Tarkanyi and Aradi 2008; Kirkpatrick et al., 2003; Herbert et al., 2001).

Therefore, inhibition of telomerase activity is an effective target for the treatment of breast cancer (Tian et al., 2010). It is investigated that curcumin(diferuloylmethane) down regulate catalytic subunit of telomerase (hTERT) (Nicole, 2008; Hsina et al., 2010). Curcumin, a polyphenol compound is derived from the herb of *Curcuma longa* (known as turmeric). It has various range of applications in medicine traditionally (Ajaikumar et al., 2008; Aggarwal et al., 2007; Sharma et al., 2005). Despite all of therapeutic application of curcumin, its utility is limited due to poor water solubility and bioavailability and high decomposition rate (Anand et al., 2008). For improving its stability and solubility, it is a good way to conjugate or encapsulate curcumin by some carrier such as cyclodextrin (Vivek et al., 2009). Commonly used cyclodextrins are α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin (Vivek et al., 2009). The

*Corresponding author. E-mail : Zarghami@tbzmed.ac.ir or Fatemeh1044@yahoo.com. Tel : +98-411-3364666. Fax: +98-411-3364666.

**Abbreviations: PCR,** Polymerase chain reaction; **MTT,** colorimetric cell viability assay; **hTERT,** telomerase; **PLGA,** poly lactic-co-glycolic acid; **DMSO,** dimethylsulfoxide.

difference between the three types is their inner cavity. The inner cavity of α-cyclodextrin is very small for curcumin loading and inner cavity of γ-cyclodextrin is too large for curcumin loading, but inner cavity of β-cyclodextrin is appropriate for curcumin loading (Vivek et al., 2009). In other nanoparticle, encapsulation technique such as poly lactic-co-glycolic acid (PLGA), only 5-10% of drug loading is possible but in cyclodextrin more than 30% of drug loading is possible (Murali et al., 2010). Although anti telomerase effect of curcumin has been studied previously in other cancer cell line (Hsina et al., 2010; Cuish et al., 2006; Jurenka et al., 2009). And preparation of β- cyclodextrin-curcumin to improve curcumin stability and solubility has been studied too (Murali et al., 2010).

Therefore, the main aim of the current work was to study the inhibitory effect of β cyclodextrin- curcumin complex on telomerase gene expression in T47D breast cancer cell line.

## MATERIALS AND METHODS

### Cell culture and cell line

T47D cell line (breast cancer epithelial like cell line), prepared from, Pasteur Institute cell bank of Iran, code:C203.this cell line were cultured in RPMI1640 (Gibco, Invitrogen,UK) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Invitrogen, UK), 2 mg/ml sodium bicarbonate,0.05 mg/ml penicillin G (Serva co, Germany), 0.08 mg/ml streptomycin (Merck co, Germany) and incubated in 37°C with humidified air containing 5% $CO_2$.

### Preparation of β-cyclodextrin-curcumin complex

β-Cyclodextrin-curcumin complex were prepared according the method of Murali et al. (2010). 40 mg of β-cyclodextrin was dissolved in 8 mL deionized water; and 12 mg of curcumin was dissolved in 500 µL acetone. These two solutions were mixed together and were placed on the stirrer at 400 rpm for 24 h without a cap to evaporate the acetone. Then, it was centrifuged at 1000 rpm for 5 min and a supernatant were collected by freeze drying.

### Determination of curcumin loading

1 mg of β-cyclodextrin-curcumin complex was dissolved in 50 mL dimethylsulfoxide (DMSO). Then, solution was placed on shaker for 24 h at room temperature. Centrifuged at 14,000 rpm and supernatant was collected for the estimations. A standard curve of curcumin concentration in DMSO was drowned by absorbance rate of curcumin via UV-vis spectrophotometer.

### *In vitro* cytotoxicity (MTT assay)

Cells in the exponential phase of growth were exposed to β-cyclodextrin-curcumin complex. Cytotoxic effect of β-cyclodextrin-curcumin complex was studied by 24, 48 and 72 h MTT assay (3, 4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide). $2 \times 10^3$ cell/well was plated in a 96-well plate (Coastar from Corning, NY) and after 24 h incubation, cells were treated with different concentrations (5-100 µM) of β-cyclodextrin-curcumin in the quadruplicate manner. Also, cyclodextrin in PBS or DMSO was used as the control. After these different exposure duration, medium was removed and then feeding of the cells with 200 µL of fresh medium. Cells were kept standing for 24 h, then 50 µL of 2 mg/ml MTT (Sigma co, Germany) was dissolved in PBS and was added to each well and plate was covered with aluminum foil and incubated for 4 h. In the next step, wells' content was removed and 200 µL pure DMSO and 25 µL Sorensen's glycine buffer was added to wells.

Finally, amount of formazan was determined measuring the absorbance at 570 nm using an ELISA plate reader (with a reference wavelength of 630 nm).

### Cell treatment

After determination of $IC_{50}$, $2.5 \times 10^5$ cells in 25 $cm^2$ flasks were treated with 3 concentrations lower than $IC_{50}$ of 24h β-Cyclodextrin-curcumin complex (5, 10 and 15 µM). Then, culture flasks were incubated for 24 h. For control cells, 1% DMSO without β-Cyclodextrin-curcumin complex was added to flask of control cells.

Then, culture flasks were incubated in 37°C containing 5% $CO_2$ with humidified atmosphere incubator for 24 h exposure duration.

### Real-time PCR (qRT-PCR) assay

In ribonucleic acid (RNA) extraction, the TRIzol (Cinnagene, Iran) was used. To prevent genomic deoxyribonucleic acid (DNA) contamination, total RNA sample were treated by DNase-I recombinant, RNase-free (Roche) as recommended protocol by manufacture. Then cDNA synthesized according to kit (First Strand cDNA Synthesis Kit fermentase, K1622).

For real-time PCR, we used hTERT primers (Genbank accession: NM_198255, bp 2165-2362) and beta actin primers (Genbank accession: NM_001101, bp 787-917). For hTERT, a 198 bp amplicon and for beta actin a 131 bp amplicon were generated in a 25 µl reaction mixture that contained: 5 pmole of the forward and reverse PCR primers of hTERT (5'CCGCCTGAGCT-GTACTTTGT3', 5' CAGGTGAGCCACGAACTGT3' respectively) or for beta actin (5'TCCCTGGAGAAGAGCTACG3', 5'GTAGTTT-CGTGGATGCCACA3' respectively), 2X PCR Master Mix Syber Green I and 2µl of the cDNA was used (Table 1).

Each DNA sample was divided so that hTERT and beta actin could be amplified,in parallel, and in duplicated from equal amounts of starting cDNA separately. 25 µl reactions contained the following final concentrations: 1X of MaximaTM SYBR Green/ROX qPCR Master Mix (including MaximaTM Hot start Taq Polymerase, MaximaTM SYBR Green qPCR Buffer, SYBR Green I and ROX Passive reference dye), 5pmole of each primer and 2µl of the cDNA. Negative controls were prepared each time, consisting of 2 µl $ddH_2O$ instead of the cDNA template.

The sample tubes were placed into the (Rotor-Gene 6000, Corbet) with the following settings as manufacture protocol (Table 2).

## RESULTS

### Determination of curcumin loading

Standard curve of curcumin concentration in DMSO was prepared via UV-Vis spectrophotometer at 450 nm (Figure 1). 1 mg of β-Cyclodextrin-curcumin complex contained 276.44 µg curcumin.

**Table 1.** Real-time PCR (qRT-PCR) assay.

| Oligonucleotide | Location | Sequence | PCR product size (pb) |
|---|---|---|---|
| **hTERT** | | | |
| Forward primer | 2165F | 5'CCGCCTGAGCTGTACTTTGT3' | 198 |
| Reverse primer | 2362R | 5' CAGGTGAGCCACGAACTGT3' | |
| **Beta-actin** | | | |
| Forward primer | 787F | 5'TCCCTGGAGAAGAGCTACG3' | 131 |
| Reverse primer | 917R | 5'GTAGTTTCGTGGATGCCACA3' | |

**Table 2.** The PCR program for hTERT and beta actin.

| Step | Temprature (°C) | Time | Number of cycles |
|---|---|---|---|
| Holding | 95 | 10 min | 1 |
| Denaturation | 95 | 15 sec | - |
| Annealing | 60 | 30 sec | 40 |
| Extension | 72 | 30 sec | - |
| Melting | 70-95 | - | 1 |

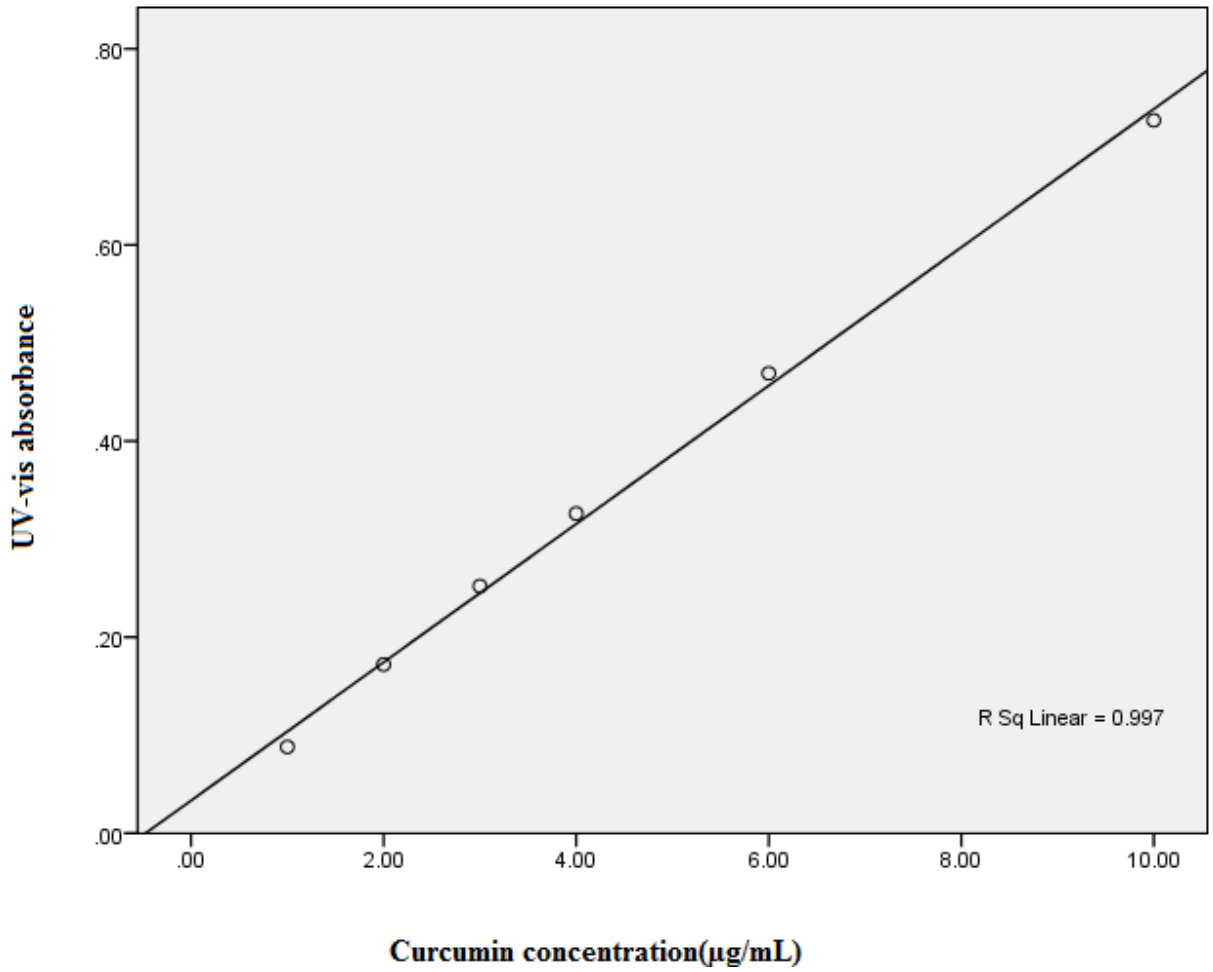


**Figure 1.** Standard plot of curcumin in DMSO solution.

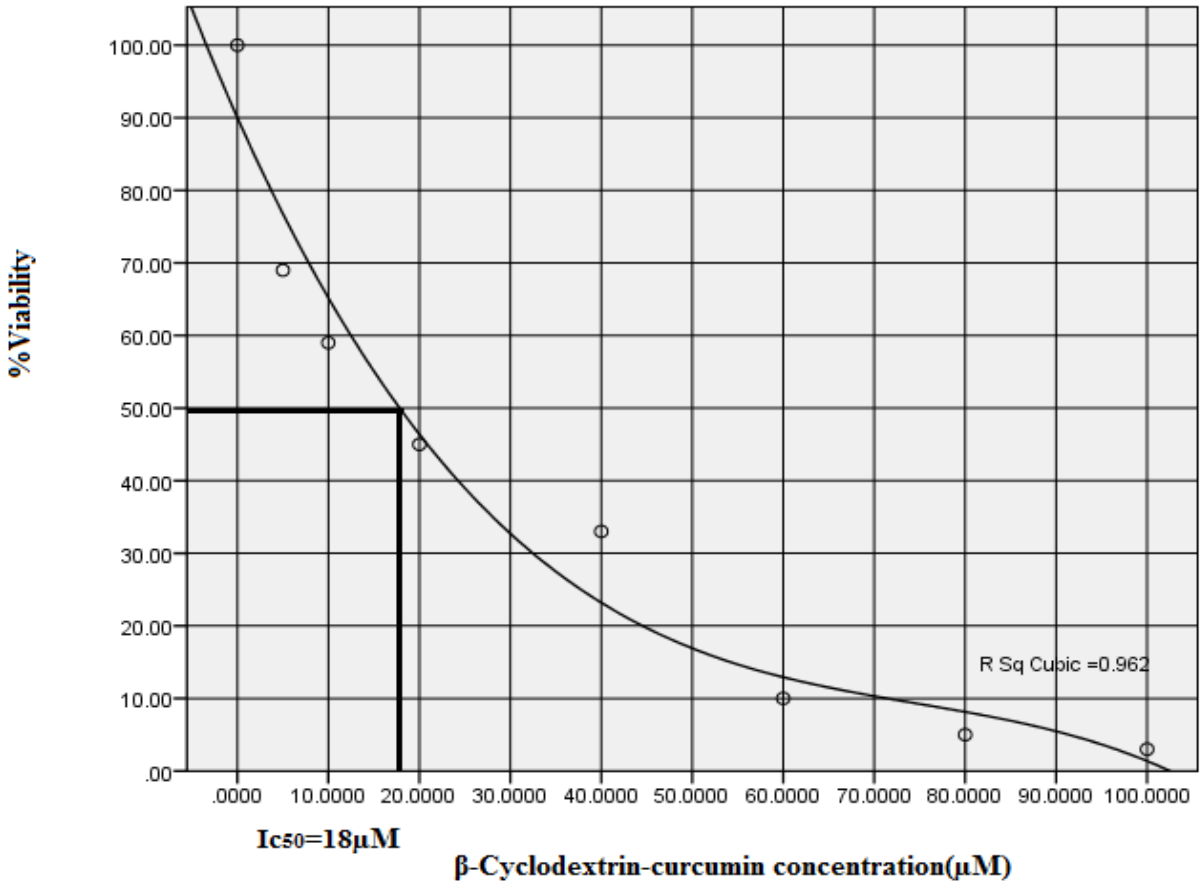


**Figure 2.** Results of MTT assay. **A)** cytotoxic effect of β-Cyclodextrin-curcumin complex on T47D cell line for 24 h. **(B)** Cytotoxic effect of β-Cyclodextrin-curcumin complex on T47D cell line for 48 h. (C) Cytotoxic effect of β-cyclodextrin-curcumin complex on T47D cell line for 72 h.

## MTT assay

T47D cell line was exposed to varying concentration of β-cyclodextrin-curcumin complex (5-100 µM) for 24, 48 and 72 h. And cell viability was demonstrated by MTT assay. β-Cyclodextrin-curcumin complex had cytotoxic effect on T47D cell line. $IC_{50}$ of 18 µMfor 24 h, 13 µM for 48 h and 11 µM for 72 h, were achieved and graph was drown by SPSS 16 (Figure 2). Results show that almost at wells with concentrations 60 -100 µM in relation to other wells, cells were dead completely.

Because our achieved $IC_{50}$s are not near to each other and showed more change, we can tell that the effect of β-cyclodextrin-curcumin complex on T47D cell line was dose-depending and time-depending.

## Cell treatment

At treatment step for the study of telomerase gene expression, we investigated telomerase gene expression at T47D breast cancer cell line after 24 h of β-cyclodextrin-curcumin complex exposure (Figure 3).

## Results for real-time PCR

hTERT mRNA levels were measured via real-time PCR. The level of hTERT mRNA was normalized to mRNA levels of the uniformly expressed housekeeping gene, beta actin, within each sample (Figure 4). The differences of $2^{-\Delta\Delta Ct}$ values were calculated. With increasing amount of $2^{-\Delta\Delta Ct}$, the expression of mRNA levels decreases (Table 3). Data analysis of real-time PCR showed that with increasing concentration of β-cyclodextrin-curcumin complex, a decreasing trend was appeared in mRNA levels of hTERT (Figure 5). Each sample was repeated 4 times.

## DISCUSSION

In this study, we used β-cyclodextrin-curcumin complex instead of free curcumin, because β-cyclodextrin enhance curcumin delivery through higher uptake by cells (Murali et al., 2010; Vivek et al., 2010). Our study demonstrated that β-cyclodextrin-curcumin complex could inhibit efficiently telomerase expression in T47D

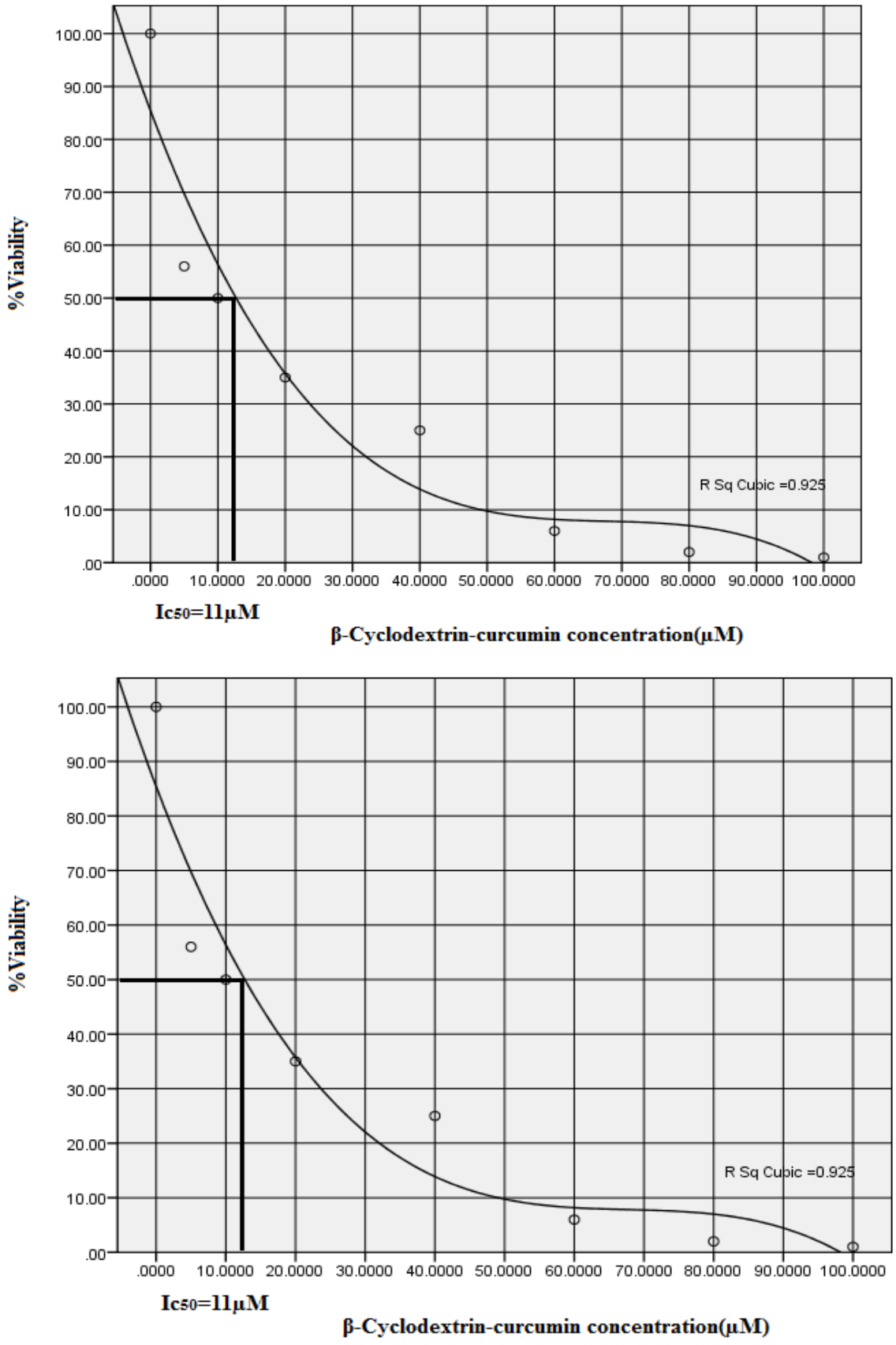


**Figure 2.** Contd.

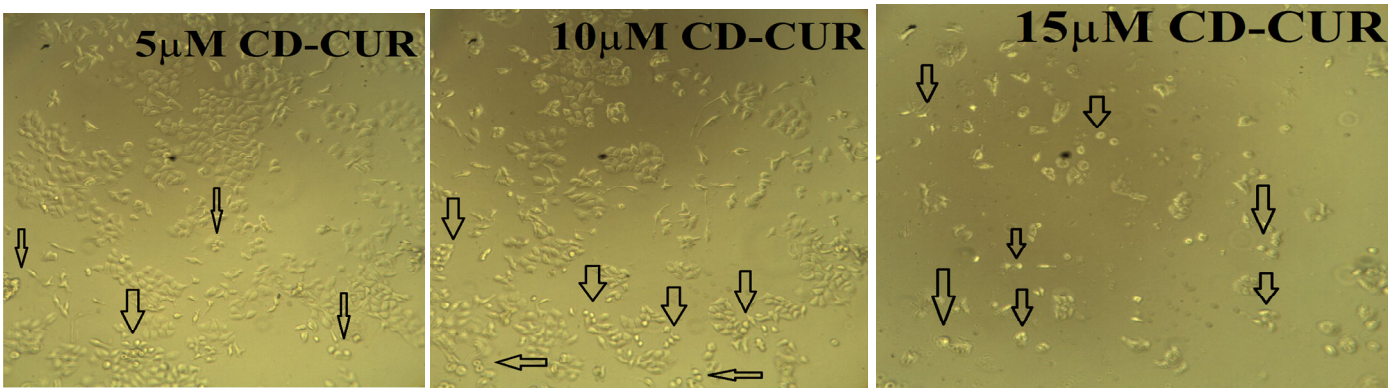


**Figure 3.** β-Cyclodextrin-curcumin (CD-CUR) treatment cells.

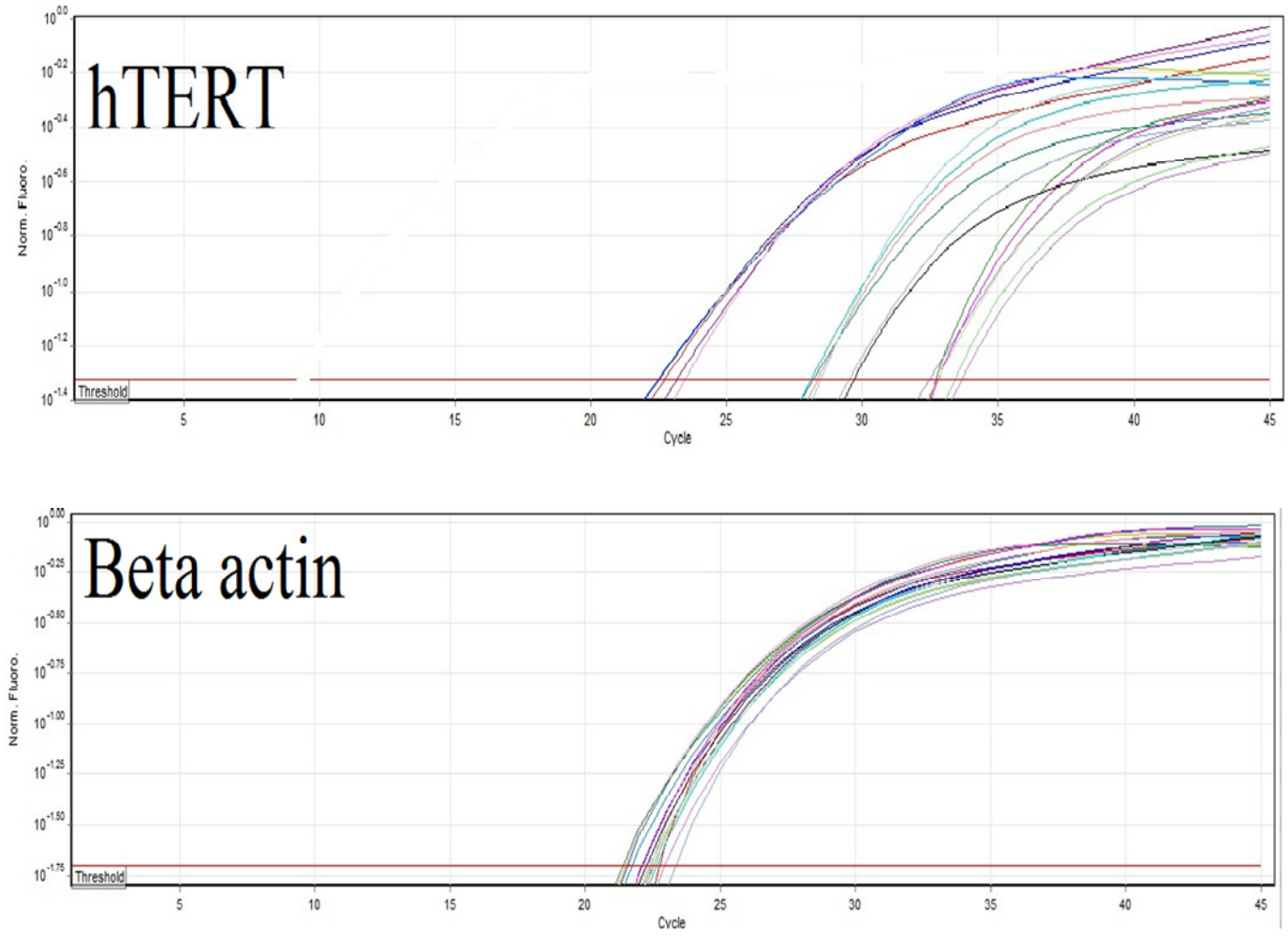


**Figure 4.** Amplification plot for hTERT and Beta actin.

breast cancer cell line. Although, curcumin has shown a wide range of pharmacological activities, its anticancer properties have attracted a great interest. The anticancer activity of curcumin has been the subject of hundreds of papers and has been reviewed in several recent articles (Ajaikumar et al., 2008; Aggarwal et al., 2007; Sharma et al., 2005; Cui et al., 2005). Despite the biological effects of curcumin, its use is limited due to: poor bioavailability,

**Table 3.** Measurement of hTERT mRNA Level in samples with ΔCt.

| Concentration of β-cyclodextrin-curcumin complex | 5 µM | 10 µM | 15 µM | Control DMSO | Control cyclodextrin | Control cell |
|---|---|---|---|---|---|---|
| Samples Ct | 23.48 | 32.76 | 33.39 | 22.51 | 23.16 | 22.72 |
| Internal Controls Ct | 22.53 | 23.55 | 23.56 | 23.12 | 23.54 | 23.52 |
| (ΔCt) | 0.95 | 9.21 | 9.83 | 0.61 | 0.38 | 0.79 |
| $2^{-\Delta\Delta Ct}$ | 0.299 | 0.001 | 0.0006 | 0.8813 | 0.7513 | 1 |

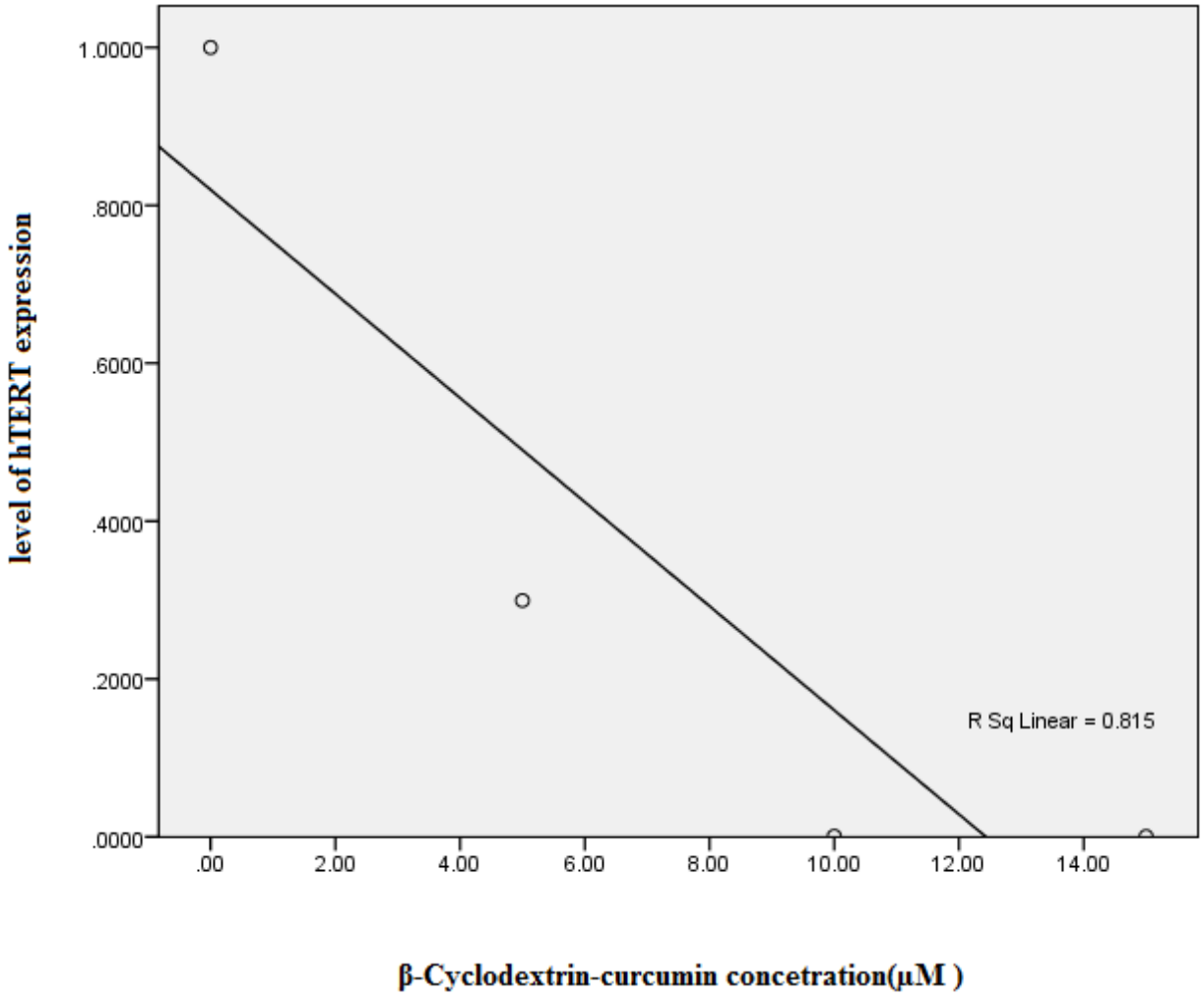


**Figure 5.** Level of hTERT mRNA expression in cells treated with β-cyclodextrin-curcumin complex.

slow dissolution rate, low water solubility, high decomposition rate in alkaline condition, photodegradation in organic solvents, and instability in the gastrointestinal tract (Rajeswari et al., 2005). For enhancing drug delivery, cyclodextrin has been used in some articles. Some examples are described.

Rajeswari et al. (2005) in their study showed cyclodextrin enhance the bioavailability of insoluble drugs by increasing the drug solubility, dissolution, and/or drug permeability. Cyclodextrin increase the permeability of insoluble, hydrophobic drugs by making the drug available at the surface of the biological barrier, skin, mucosa, or the eye cornea, from where it partitions into the membrane without disrupting the lipid layers of the barrier.

Vivek et al. (2010) and in their study showed cyclodextrin-complexed curcumin had superior attributes compared with free curcumin for cellular

uptake and for antiproliferative and antiinflammatory activities.

Parallel with our study, to evaluate cytotoxic effect of curcumin and β-cyclodextrin-curcumin complex on the proliferation of prostate cancer cells, Murali et al. (2010) treated DU145 and C4-2 cells with (5-40 µM) curcumin and β-Cyclodextrin-curcumin complex, then detected the cell viability by the MTS method. β-Cyclodextrin-curcumin complex inhibited the growth of prostate cells higher than free curcumin.

According to our study, to evaluate cytotoxic effect of β-cyclodextrin-curcumin complex on proliferation of T47D breast cancer cell line, we treated this cell line with different concentration (5-100µM) of β-cyclodextrin-curcumin complex for three times 24, 48 and 72 h by MTT assay. For this purpose, we prepared β-cyclodextrin-curcumin complex according to the method of (Murali et al., 2010), the proportion of curcumin to cyclodextrin was 30%. In other words, approximately about 30% of curcumin loaded into cyclodextrin. Because Murali et al. (2010) in their study showed that in this proportion, curcumin effectively was uptake from cells, we investigated that curcumin-cyclodextrin had anti cancer effect onT47D breast cancer cell line.

It should be noted that the effect of β-cyclodextrin-curcumin complex in T47D cell line has never been done so far, and inhibitory effect of β-cyclodextrin-curcumin complex on hTERT expression gene has never been done so far. So there isn't anything for comparison. The *in vitro* effect of β-cyclodextrin-curcumin complex on T47D cell line requires further investigation. But studies in other cancer cell lines have shown that β-cyclodextrin-curcumin complex were more effective than free curcumin in the cancer cell lines.

## Conclusion

Our results show that β-cyclodextrin-curcumin complex had inhibitory effect on breast cancer T47D cell line. This inhibition was dose-dependent and time-dependent too. Cytotoxic effect of β-cyclodextrin-curcumin complex in the cells was increased with increasing concentration of β-cyclodextrin-curcumin complex. Data analysis showed that with increasing concentration of β-Cyclodextrin-curcumin complex, decreasing trend of telomerase expression was observed. Briefly, as our data showed that cyclodextrin-curcumin inclusion complex had inhibitory effect on expression of hTERT mRNA, we can use this complex as an anti cancer drug in breast cancer treatment.

## ACKNOWLEDGEMENTS

This study was supported by grants from Drug Applied Research Center of Tabriz University of Medical Sciences. We thank thereby Drug Applied Research Center of Tabriz University of Medical Sciences for funding this research.

## REFERENCES

Aggarwal BB, Sundaram C, Malani N, Ichikawa H (2007). Curcumin: the Indian solid gold. Adv. Exp. Med. Biol. 595: 1-75.

Ajaikumar B, Kunnumakkara P, Anand B, Aggarwal B (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Letters, 269: 199-225.

Anand P (2008). Biological activatives of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharma. 76: 1590-1611.

Cui SH (2006).Curcumin inhibits telomerase activity in human cancer cell lines. Int. J. Mol. Med. 18: 227-231.

Cui SX, Qu XJ, Xie YY, Zhou L, Nakata M, Makuuchi M, Tang W(2006). Curcumin inhibits telomerase activity in human cancer cell lines. Int. J. Mol. Med. p. 18.

Herbet B, Wright WE, Shaly J (2001).Telomerase and breast cancer. Breast cancer Res. 3(3): 146-149.

Hsina I, Sheua G, Chena H, Chiua L, Wange W, Chana H, Hsuc CH, Liang D (2010). N-acetyle cysteine mitigates curcumin-mediated telomerase inhibition through rescuing of SP1 reduction in A549 cells. Mut. Res. 688: 72-77.

Jurenka J (2009). Anti-inflammatory properties of curcumin, a major constituent of curcuma longa: A review of preclinical and clinical research. Alter. Med. Rev. 14(2): 141-152.

Kirkpatrick KL, Ogunkolade W, Elkak AE, Bustin S, Jenkins P, Ghilchick M, Newbold RF, Mokbel K (2003). hTERT expression in human breast cancer and noncancerous breast tissue: correlation with tumour stage and c-Myc expression. Breast Cancer Res. Treatment, 77: 277-284.

Murali MY, Jaggi S, Chauhan C (2010). β -Cyclodextrin-curcumin self-assembly enhances curcumin delivery in prostate cancer cells. Colloids and Surfaces B: Biointerfaces, 79: 113-125.

Nicole MB (2008). Targeting telomerase and telomeres with small molecule anti cancer compounds. MMG 445 Basic Biotechnol. J. 4: 72-79.

Rajeswari C, Alka A, Javed A, Khar RK (2005). Cyclodextrins in Drug Delivery: AAPS Pharm. Sci. Tech. 6(2): p. 43.

Sharma RA, Gescher AJ, Steward WP (2005). Curcumin: the story so far. Eur. J. Cancer, 41(13): 1955-1968.

Tarkanyi I, Aradi J (2008). Pharmacological intervention strategies for affecting telomerase activity. Future prospects to treat cancer and degenerative disease. Biochimie. 90: 156-172.

Tian N, Gaines WJ, Benjamin ZF (2010). Female breast cancer mortality clusters within racial groups in the United States. Health Place, 16: 209-218.

Vivek RY, Sarasija S, Kshama D, Seema Y (2009). Effect of Cyclodextrin Complexation of Curcumin on its Solubility and Antiangiogenic and Anti-inflammatory Activity in Rat Colitis Model. AAPS. Pharm. Sci. Tech. 10(3).

Vivek RY ,Sahdeo P, Ramaswamy K, Jayaraj R, Madan MC, Lauri V, Jaakko P, Bharat BA (2010). Cyclodextrin-complexed curcumin exhibits anti-inflammatory and antiproliferative activities superior to those of curcumin through highercellular uptake. Biochem. Pharmacol. 80: 1021-1032.