The inhibitory effect of *Curcuma longa* extract on telomerase activity in A549 lung cancer cell line

Pourhassan Mohammad, Zarghami Nosratollah*, Rahmati Mohammad, Alibakhshi Abbas and Ranjbari Javad

Department of Clinical Biochemistry and Laboratory Medicine, Division of Medical Biotechnology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran and Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

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Telomerase is reactivated in lung cancer cells, the most prevalent cancer worldwide, but not normal cells. Therefore, targeting it, preferably with natural compounds derive from medicinal plant such as curcumin, could have important effect on treatment of lung cancer. Curcumin, derived from *Curcuma longa* rhizome, has many anti-cancer activities. Therefore, the main objective of current work was to study inhibitory effect of *C. longa* total extract on telomerase in A549 lung cancer cell line as *in vitro* model of lung cancer. First, total extract of *C. longa* were prepared by n-hexane, methanol and dichloromethane. Then, cytotoxic effect of n-hexane phase was studied on A549 lung cancer cell line with 24, 48 and 72 h MTT assays. Finally, after determination of IC50, cells were treated with n-hexane extract and TRAP (Telomeric Repeat Amplification Protocol) assay was done to measure amount of relative telomerase inhibition by the extract. Data analysis showed that n-hexane extract of *C. longa* has dose-dependent cytotoxic effect on A549 lung cancer cell line with IC50 = 0.23 - 0.28 mg/ml and the extract inhibits telomerase activity with dose-dependent manner. In conclusion, n-hexane extract of *C. longa* has cytotoxic and telomerase-inhibitory effect on cell line A549 and could be exploited as potential source for developing novel drugs against lung cancer.

**Key words:** Telomerase, lung cancer, *Curcuma longa*, A549 lung cancer cell line.

**INTRODUCTION**

Telomerase is a ribonucleoprotein reverse transcriptase which maintains telomeric ends of eukaryotic chromosomes during DNA replication. It has been shown that telomerase is active in more than 85% of cancers including lung cancer but not normal cells (Lingner et al., 1995; Kim, 1997). On the other hand, lung cancer is the most prevalent cancer worldwide (Jemal et al., 2007). Therefore, targeting the telomerase in this cancer could be promising step in its treatment. Different agents have been proposed for telomerase inhibition (Strahl and Blackburn, 1996; Damm et al., 2001; Zou et al., 2006; Massard et al., 2006; Nakamura et al., 2005; Mizushima et al., 2000; Yeo et al., 2005). But, they have undesirable complications (Mittal et al., 2004) and it is necessary to seek alternative telomerase-inhibiting agents, preferably natural compounds such as curcumin.

*Curcuma longa* is a medicinal plant which its constituents, especially curcumin, has diverse anti-cancer properties including anti-telomerase action (Sanjay et al., 2007; Anand et al., 2008). Anti-telomerase activity of pure curcumin has been studied previously in some cancer cells other than lung cancer cells (Cui et al., 2006; Chakraborty et al., 2006; Ramachandran et al., 2002; Singh and Singh, 2009; Mukherjee et al., 2007). However, to date any study for investigating the effect of curcuma total extract on telomerase in the lung cancer cells remains unperformed. Therefore, the main aim of current work was to study the effect of *C. longa* total extract on telomerase activity in A549 lung cancer cell line as *in vitro* model of lung cancer for its possible therapeutic application in the future.
**MATERIALS AND METHODS**

**Extract preparation**

100 g powder of *C. longa* rhizome was dissolved in 200 ml n-hexane and solution was shaken for 4 h at 45°C. Then, supernatant was transferred to a tube. This step was performed three times. In the next step, residue of n-hexane extraction was dissolved in 200 ml dichloromethane instead of n-hexane and same steps repeated using dichloromethane. In the next step, debris of previous extraction step with dichloromethane was dissolved in 200 ml methanol and at the same way supernatant was collected. Finally, solvents of all three phases were dried by rotatory-evaporator and remained powders were stored at -20°C until used.

**Selection of appropriate phase**

Based on the fact that Curcuminoids have maximum absorbance at $\lambda_m = 420 - 430$ nm (Jayaprakasha et al., 2005), the curcuminoids content of n-hexane, dichloromethane and methanol phases of extract was determined spectrophotometrically. Briefly, ODs of 0.038, 0.0304, 0.019, 0.0152, 0.0076 and 0.0019 mg/ml solutions of pure curcumin (Sigma) dissolved in 10% DMSO and ODs of 0.0038 mg/ml solutions of extract phases in 10% DMSO were determined in $\lambda_m = 420$ nm and $\lambda_m = 430$ nm with three times reading in the spectrophotometer. Then, standard curve was plotted using mean of pure curcumin OD×100. Finally, relative curcumin content of phases was determined according to standard curve.

**Cell culture and cytotoxicity**

A549 lung cancer cell line (kindly dedicated from pharmaceutical nanotechnology research center, Tabriz University of Medical Sciences, Tabriz, Iran) 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$_2$. After growing sufficient amount of cells, cytotoxic effect of n-hexane extract was studied as follows: 24, 48 and 72 h MTT assays (Carmichael et al., 1987). Briefly, 1000 cell/well were cultivated in a 96 well plate. After 24 h incubation in 37°C with humidified atmosphere containing 5% CO$_2$, cells were treated with serial concentrations of n-hexane extract of *C. longa* (0 mg/ml to 0.57 mg/ml) for 24, 48 and 72 h in the quadruplicate manner as cells which received 0 mg/ml extract + 200 µl culture medium containing 10% DMSO served as control. After incubation, the medium of all wells of plate were exchanged with fresh medium and cells were harvested for 24 h in incubator. Then, medium of all wells were removed carefully and 50 µl of 2 mg/ml MTT (Sigma co, Germany) dissolved in PBS was added to each well and plate was incubated for 2 h at room temperature. Then, module wells were washed three times and horseradish peroxidase-conjugated anti-digoxigenin antibody was added to wells. After that, substrate solution containing 3′,5′ tetra methyl benzene (TMB) was added to wells, wells incubated in room temperature for 20 min for color development and more color development was stopped. Finally, absorbance of developed blue color was measured at 450 nm by STAT-FAX 2100 ELISA reader (with a reference wavelength of 630 nm).

**Data analysis**

For data analysis of MTT assays, mean OD of each well was calculated. Then, OD of tests and controls was corrected by subtracting mean OD of tests and controls from mean OD of blanks (medium alone). Then, percent of viability of control was calculated according to this formula: percent of viability of control = corrected mean OD of tests / corrected mean OD of controls × 100. Finally, graph of results was plotted using SPSS 16.0 and IC50 (half maximal inhibitory concentration) of n-hexane extract on A549 was calculated.

Relative telomerase activity percent (%RTA) of each sample was calculated according to instructions of TeloTAGGG Telomerase PCR ELISA PLUS kit. Then, %RTA of control cell was calculated for each sample and related graph was plotted using SPSS 16.0.

**RESULTS**

Data analysis of spectrophotometric measurement of extracted phases curcuminoids content showed that n-hexane phase has more curcumin than methanol and dichloromethane phases and dichloromethane phase has the lowest content of curcuminoids (Figure 1 and Table 1). Therefore, we investigated the effect of n-hexane extract...
Figure 1. OD\textsubscript{420} and OD\textsubscript{430} of *Curcuma longa* extracts in comparison with pure curcumin (as standard).

Table 1. Mean absorbances of pure curcumin, n-hexane extract, methanol extract and dichloromethane extract solutions in λ\textsubscript{max} = 420 nm and λ\textsubscript{max} = 430 nm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pure curcumin (as standard)</th>
<th>n-hexane extract</th>
<th>Methanol extract</th>
<th>Dichloromethane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (mg/ml)</td>
<td>0.0019</td>
<td>0.0076</td>
<td>0.0152</td>
<td>0.0038</td>
</tr>
<tr>
<td>λ\textsubscript{max} = 420 nm</td>
<td>0.0025</td>
<td>0.0049</td>
<td>0.0108</td>
<td>0.0097</td>
</tr>
<tr>
<td>λ\textsubscript{max} = 430 nm</td>
<td>0.0024</td>
<td>0.0050</td>
<td>0.01055</td>
<td>0.0096</td>
</tr>
<tr>
<td>Mean OD\times100</td>
<td>0.245</td>
<td>0.492</td>
<td>1.0675</td>
<td>0.965</td>
</tr>
</tbody>
</table>

of *C. longa* on telomerase activity in the A549 cell line. Data analysis of cytotoxicity assay showed that IC\textsubscript{50} of n-hexane extract on A549 lung cancer cell line is 0.28, 0.27 and 0.23 mg/ml for 24, 48 and 72 h MTT assays, respectively (Figure 2). Figure 2 shows that IC\textsubscript{50} of n-hexane extract on A549 lung cancer cell line is dose-dependent but not time dependent. Therefore, we investigated telomerase-inhibitory effect of n-hexane extract on A549 for 24 h exposure time (Figure 3). Morphological analysis showed no significant difference between control and treated cells.

Data analysis obtained from TRAP assay showed that n-hexane extract of *C. longa* inhibits telomerase activity dose-dependently and doses higher than 0.114 mg/ml completely inhibit telomerase relative activity (Figure 4, Tables 2 and 3). Table 2 shows that concentration of 0.142 mg/ml is the least effective dose for complete inhibition of telomerase activity in A549 lung cancer cell line. In addition, in this dose telomerase activity was inhibited 46.30 folds in comparison with untreated cells. Therefore, dose of 0.142 mg/ml may be a cut-off point for telomerase inhibition by n-hexane extract in the A549 cell
Figure 2. Cytotoxic effect of n-hexane extract on A549 lung cancer cell line after 24 h (A), 48 h (B) and 72 h (C) exposure. (D). IC50 of n-hexane extract of Curcuma longa on A549 tumor cell line after 24, 48 and 72 h treatment.
**DISCUSSION**

In the present study, total extract used instead of pure curcumin. Because, beside synergistic effects of curcuminoids on each other in a solution (Deshpande and Maru, 1995) and based on Jayaprakasha et al. (2005) report, extraction of pure curcumin from rhizome of *C. longa* is time consumable and needs for using HPLC methods.

In the current work, MTT assay showed that n-hexane extract of *C. longa* has dose-dependent but not time-dependent cytotoxicity on the A549 lung cancer cell line (IC50 = 0.23 to 0.30 mg/ml). This finding is in accordance with result of a study (Radhakrishna et al., 2004) in which pure curcumin had dose-dependent but not time-dependent cytotoxicity on A549, but IC50 of pure curcumin was 0.01841 mg/ml (50 μM) for 24 h exposure in that study. In addition, results of same study showed IC50 = 0.01474 mg/ml (40 μM) of pure curcumin on H1299 cells (Radhakrishna et al., 2004). This difference in the IC50 may be due to purity of curcumin and existence of other substances other than curcumin which may interfere with action of curcumin. Therefore, there is need for separation and further study of n-hexane extraction constituents in the future. Shi et al. (2006) showed that 40 μM curcumin inhibits growth of the human epithelium ovarian cancer cell line Ho-8910 in vitro by 52% for 48 h exposure (P < 0.05) (Shi et al., 2006). However, results of current work demonstrated that IC50 of *C. longa* extract

![Control cells](image1.png) ![Treated with 0.114 mg/ml extract](image2.png) ![Treated with 0.142 mg/ml extract](image3.png)

![Treated with 0.171 mg/ml extract](image4.png) ![Treated with 0.199 mg/ml extract](image5.png)

**Figure 3.** Morphological effect of n-hexane extract of *Curcuma longa* on A549 lung cancer cell line after 24 h treatment.
Table 2. Determination of telomerase activity in A549 tumor cells treated with different concentrations of n-hexane extract of Curcuma longa.

<table>
<thead>
<tr>
<th>Dose (mg/ml)</th>
<th>Telomerase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>+</td>
</tr>
<tr>
<td>0.028</td>
<td>+</td>
</tr>
<tr>
<td>0.057</td>
<td>+</td>
</tr>
<tr>
<td>0.114</td>
<td>+</td>
</tr>
<tr>
<td>0.142</td>
<td>–</td>
</tr>
<tr>
<td>0.171</td>
<td>–</td>
</tr>
<tr>
<td>0.199</td>
<td>–</td>
</tr>
</tbody>
</table>

*The cut off point of being positive (+) and negative (-) was calculated based on following formula:
OD of sample - OD of negative control = A
If A > 2× (OD of negative control), then, telomerase activity considered positive and if not so, activity considered negative.

is about 0.26 mg/ml in A549 lung cancer cell line. This different finding may be is due to different behavior of two cells in response to curcumin and shows that these cancers may differently response to exposure with curcumin in vivo.

Currently there are different types of standard treatment methods for lung cancer patients such as surgery, radiation, chemotherapy, laser therapy, photodynamic therapy (PDT), biologic therapy and gene targeted therapy (Thatcher, 2008). However, there are harsh side effects about all (Geamanu, 2008). Therefore, with regarding to these undesirable effects and result of current study, it seems that C. longa may be an appropriate source for developing novel drugs against lung cancer and can be a safer alternative for current treatment regimens.

In conclusion, results of current study showed that n-hexane extract of C. longa has inhibitory effect on telomerase in the A549 lung cancer cell line. Inhibition of telomerase by n-hexane extract of C. longa in these cells is reported for the first time and even any study, in which
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REFERENCES


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