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Cytotoxicity screening of curcumin on HeLa cancer cell lines

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Abstract
Phytocompounds are most extensively studied as an alternative approach in cancer therapeutics. Curcumin is one of the leading compounds from turmeric that has been most extensively studied for its promising pharmacological effects. In the present study, the cytotoxicity potential of curcumin was determined by MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide] assay on HeLa cancer cell lines. The MTT assay was conducted at 48h incubation to determine percent cell viability. The IC_{50} values of curcumin was 3.36µM. The curcumin showed dose-dependent cytotoxicity on cervical epithelioid adenocarcinoma cells. The *in vitro* cytotoxicity potential of curcumin in terms of percent cell viability proved to be more effective on HeLa cells.

Keywords: Curcumin, cytotoxicity, MTT, IC_{50}, HeLa

1. Introduction
Cancer is a multistage disease characterized by uncontrolled and unregulated cellular growth [1, 2]. Recently, much attention has been focused on phytocompounds for combating major challenges in cancer therapy such as drug resistance, metastasis, and recurrence. The 74% of all drugs which are approved in cancer therapeutics from 1981 to 2002, were derived either from natural compounds or based on natural compounds [3]. Amongst various natural compounds, polyphenols are primarily involved in regulating cancer signaling pathways [4, 5, 6], inhibiting cancer cell proliferation resulting in tumor regression. Curcumin is a natural polyphenol obtained from the rhizome of *Curcuma longa* of the Zingiberaceae family [7, 8]. It has been used extensively from ancient time in the treatment of many diseases [9, 10]. It is widely available, non-toxic, and possesses different pharmacological activities such as antibacterial, antioxidant, antiviral, analgesic, anticancer, antifungal and anti-inflammatory [11, 12, 13, 14]. The anticancer effects of curcumin are important because it prevents the proliferation of cancer cells but does not harm healthy cells [15]. In the current study, an investigation was undertaken to determine the cytotoxicity of curcumin on HeLa cancer cell lines.

2. Materials and Methods
2.1 Materials
Curcumin (#C1386), Dimethly sulfoxide (#D8418) were purchased from Sigma-Aldrich Chemical Co. Ltd. Dulbecco's Modified Eagle's Medium (GE Healthcare Life Sciences, #SH30243.01), Dulbecco's phosphate-buffered saline (GE Healthcare Life Sciences, #SH30028.02). Trypan blue, absolute ethanol, MTT [3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide], trypsin, amphotericin, penicillin, streptomycin, fetal bovine serum, (Hi-Media Labs, Mumbai, India) and other reagents of analytical grade were also used in the study.

2.2 Cell lines and culture conditions
The HeLa cancer cell lines (National Centre for Cell Science, Pune, India) were used for *in vitro* cytotoxicity assay. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (4.5g/L Glucose), supplemented with L-glutamine (4mM), penicillin 10,000 units/mL, 10% heat-inactivated fetal bovine serum (FBS), streptomycin 1000µg/mL and Amphotericin-B solution 25µg/ mL.

The cells were incubated at 37 °C in a humidified Atmosphere of 95 percent air and 5 percent CO_2. The adherent cells were detached using trypsin-EDTA solution 1X/0.25% after 24h of incubation. The Trypan blue dye exclusion method was followed for cell counting using the Luna automated cell counter (Logos Bio systems, USA).

2.3 Determination of cytotoxicity

The cytotoxicity of curcumin on HeLa cancer cell lines was determined by MTT (3-(4, 5-dimethylthiazolyl -2)-2, 5-diphenyltetrazolium bromide) assay. The cell suspension of 200µL was seeded in 96-well micro plates at a density of 20,000 cells/well and incubated in 5% CO_2 incubator at 37 °C for 24h, after which the cells were exposed to a graded concentration of 3.125, 6.25, 12.5, 25 and 50µM of curcumin and incubated for 48h. Later media was removed from all treated and vehicle control (1% DMSO) cells and replaced with fresh media containing MTT (0.5 mg/ml) and were re-incubated for 3h at 37 °C. The MTT solution was removed and then the 100µL of DMSO was added to dissolve insoluble formazan crystals formed within the mitochondria of viable cells. The plate was incubated with DMSO for 5min with gentle shaking. The cell viability was determined by measuring the absorbance on a micro plate reader (ELx800, Bio Tek, USA) at λ max 570 nm. A dose-response curve was constructed and the concentration of curcumin that resulted in 50 percent inhibition of cell growth was calculated as the half-maximal inhibitory concentration (IC_{50}). The IC_{50} value was determined by a linear curve fit generated after obtaining dose-response to five different concentrations of curcumin. The cell viability percentages (y-axis) were plotted against increasing concentrations of curcumin on the x-axis. The IC_{50} values were estimated by using the linear regression equation

$$y = mx + c$$

Where 50 is substituted for y, yielding x as the IC_{50} value.

$$\text{Cell Viability (\%)} = \frac{\text{Number of cells treated with the test item}}{\text{Number of untreated cells}} \text{X } 100$$

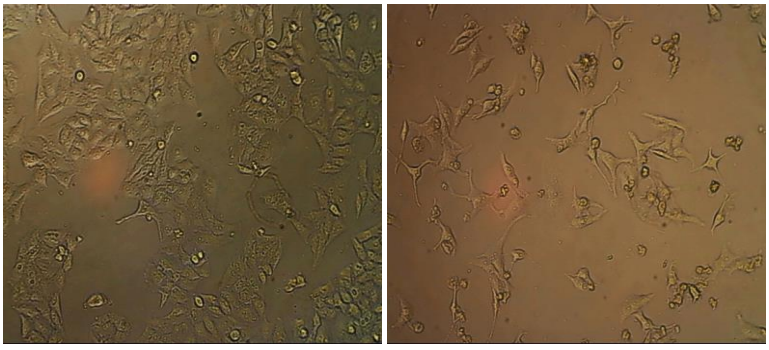
2.4 Statistical analysis

The MTT assay data was subjected to linear regression analysis to obtain dose-response curves and IC_{50} values. The student t-test was carried out and all the values were expressed as Mean± SD (Graph Pad Prism, Version 5).

3. Results and Discussion

3.1 *In vitro* cytotoxicity assay

The IC_{50} value of curcumin on HeLa cells was 3.36µM, (Fig.1b). The proliferation of HeLA cells was significantly (p<0.0001) inhibited by curcumin in a dose-dependent manner at 48h incubation (Fig.2). The treated HeLa cells with curcumin showed shrinkage and partial detachment, thus suggesting cytotoxicity of curcumin at 48h incubation (Fig.1b). The findings of our study indicated that curcumin in terms of percent cell viability on HeLa cell lines showed dose-dependent cytotoxicity [16, 17]. In conclusion, the successful results of the present study along with the additional set of investigations could make way the possibility of a combination of the curcumin as an adjuvant with the available conventional drugs to encounter drug resistance and adverse effects in cervical cancer.



a b

Fig 1: Representative Images of cytotoxic effect of a) vehicle control b) curcumin on HeLa cell lines at 48h incubation (10X)

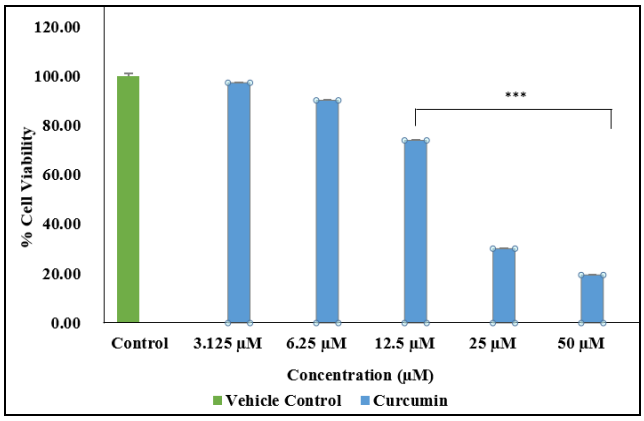


Fig 2: Histogram depiction of dose dependent cytotoxicity data of curcumin as determined by MTT assay on HeLa cell lines. Values are Mean ± SD, n=3, (***p<0.0001)

4. Acknowledgement

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