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Evaluation of curcumin for cytotoxicity potential on MDA-MB-231 cancer cell lines

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Abstract
Natural compounds have gained a promising role in both cancer prevention and therapy. They play an important role in the discovery of lead compounds and represent an excellent source of novel anticancer drugs. Curcumin is a renowned natural compound derived from the rhizome *Curcuma longa* L, commonly known as turmeric. In the present study, the cytotoxicity potential of curcumin on MDA-MB-231 cell lines was determined by MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay. It was conducted at 48h incubation to determine percent cell viability. The IC_{50} value of curcumin was 18.54µM. The curcumin showed dose-dependent cytotoxicity on triple-negative breast cancer cells. The *in vitro* cytotoxicity potential of curcumin in terms of percent cell viability proved to be relatively effective on triple-negative breast cancer cells.

Keywords: Curcumin, cytotoxicity, MTT, IC_{50}, MDA-MB-231

1. Introduction
Drug resistance, metastasis, and recurrence remain a significant challenge in cancer therapy [1], despite of various therapeutic strategies such as chemotherapy, hormone therapy, immunotherapy, radiation therapy, targeted therapy, gene therapy [2, 3]. Natural compounds have attracted the attention of scientific community for the development of antitumor drugs, due to their proven efficacy and safety. Due to their anti-proliferative and pro-apoptotic properties, they have been recently introduced as anti-cancer adjuvant therapies [4]. Curcumin is one of the most important natural compound, as it possess pleiotropic [5] antitumor properties, sensitizes tumor cells to targeted therapeutic agents, reverses resistance to chemotherapeutic drugs [6, 7], exhibits anti-metastatic property by inhibiting transcription factors, protein kinases, proteases, inflammatory cytokines, and their signaling pathways [1, 8, 9]. It was extracted in a pure crystalline form for the first time from turmeric plant in 1870 [10]. It possesses a wide range of pharmacological properties such as anti-inflammatory [11], anticancer [12, 13], anti-oxidant [14], antimicrobial [15], wound healing [16], antimalarial [17], neuron protective, antidiabetic [18]. In the current study, an investigation was undertaken to determine the cytotoxicity of curcumin on MDA-MB-231 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], streptomycin, penicillin, amphotericin, trypsin, 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 *in vitro* cytotoxicity assay was carried out on MDA-MB-231 cancer cell lines from NCCS (National Centre for Cell Science), India. The Dulbecco's Modified Eagle's Medium (DMEM) (4.5g/L Glucose), supplemented with 10% heat-inactivated fetal bovine serum (FBS),

Penicillin 10,000 units/mL, Amphotericin-B solution 25µg/mL, streptomycin 1000µg/mL and L-glutamine (4mM) was used. 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 MDA-MB-231 cancer cell lines was determined by MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. The MDA-MB-231 cells were seeded at a density of 20,000 cells/well in 96-well micro plates 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.

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

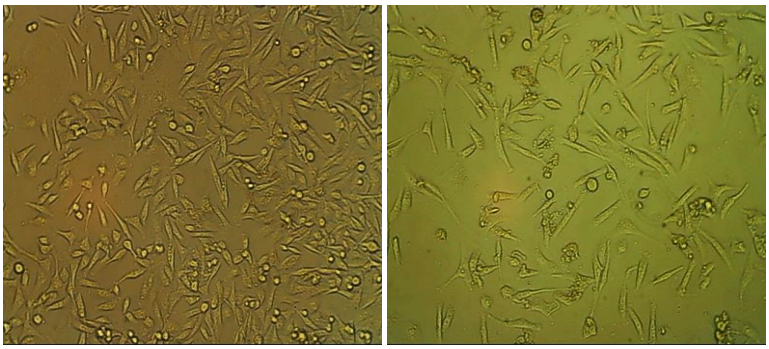
2.4 Statistical analysis

The *in vitro* cytotoxicity study 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 proliferation of MDA-MB-231 cells was significantly (p<0.0001) inhibited by curcumin in a dose-dependent manner at 48h incubation (Fig.2). The IC_{50} value of curcumin on MDA-MB-231 was 18.54µM, (Fig.1b). The treated MDA-MB-231 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 MDA-MB-231 cell lines showed dose-dependent cytotoxicity [19, 7]. 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 triple-negative breast cancer.



a b

Fig 1: Representative images of cytotoxic effect of a) vehicle control b) curcumin on MDA-MB-231 cell lines at 48h incubation (10X)

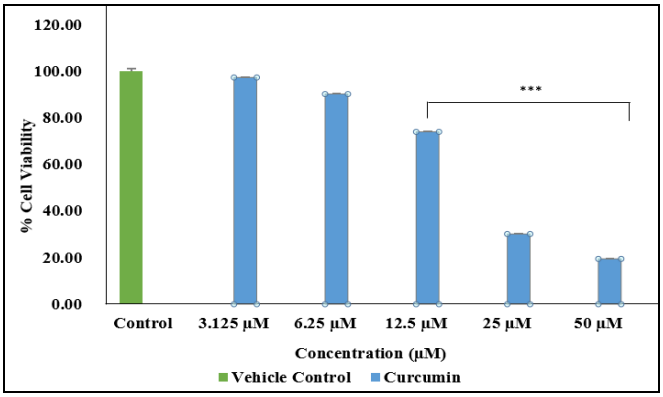


Fig 2: Histogram depiction of dose dependent cytotoxicity data of curcumin as determined by MTT assay on MDA-MB-231 cell lines. Values are Mean ± SD, n=3, (***p<0.0001)

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5. References

1. Koroth J, Nirgude S, Tiwari S, Gopalakrishnan V, Mahadeva R, Kumar S *et al*. Investigation of anti-cancer and migrastatic properties of novel curcumin derivatives on breast and ovarian cancer cell lines. BMC Complementary and Alternative Medicine. 2019; 19:273.
2. Arruebo M, Vilaboa N, Saez-Gutierrez B, Lambea J, Tres A, Valladares M *et al*. Assessment of the evolution of cancer treatment therapies. Cancers. 2011; 3:3279-330.
3. Urruticoechea A, Alemany R, Balart J, Villanueva A, Vinals F, Capella G *et al*. Recent advances in cancer therapy: an overview. Current Pharmaceutical Design.

2010; 16:3-10.
4. Pucci C, Martinelli C, Ciofani G. Innovative approaches for cancer treatment: current perspectives and new challenges. Cancer. 2019; 13:961.
5. Anand P, Sundaram C, Jhurani S. Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Letters. 2008; 267(1):133-64.
6. Zhou QM, Wang XF, Liu XJ, Zhang H, Lu YY, Huang S *et al*. Curcumin improves MMC-based chemotherapy by simultaneously sensitizing cancer cells to MMC and reducing MMC-associated side-effects. European Journal of Cancer. 2011; 47:2240-2247.
7. Hu S, Xu Y, Meng L, Huang L, Sun H. Curcumin inhibits proliferation and promotes apoptosis of breast cancer cells. Experimental and Therapeutic Medicine. 2018; 16:1266-1272.
8. Xu YX, Pindolia KR, Janakiraman N, Noth CJ, Chapman RA, Gautam SC *et al*. Curcumin, a compound with anti-inflammatory and anti-oxidant properties, down-regulates chemokine expression in bone marrow stromal cells. Experimental Hematology. 1997; 25:413-422.
9. Deng Y, Verron E, Rohanizadeh R. Molecular mechanisms of anti-metastatic activity of curcumin. Anticancer Research. 2016; 36:5639-5647.
10. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curcumin": From kitchen to clinic. Biochemical Pharmacology. 2008; 75:787-809.
11. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. Altern Medicine Review. 2009; 14(2):141-153.
12. Salem M, Rohani S, Gillies ER. Curcumin, a promising anti-cancer therapeutic: A review of its chemical properties, bioactivity and approaches to cancer cell delivery. RSC Advances. 2014; 4(21):10815-10829.
13. Bachmeier BE, Killian PH, Melchart D. The role of curcumin in prevention and management of metastatic disease. International Journal of Molecular Sciences. 2018; 19:1716.
14. Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radical Biology and Medicine. 2000; 28(8):1303-1312.
15. De R, Kundu P, Swarnakar S. Antimicrobial activity of curcumin against *Helicobacter pylori* isolates from India and during infections in mice. Antimicrob Agents and Chemotherapy. 2009; 53(4):1592-1597.
16. Phan TT, See P, Lee ST, Chan SY. Protective effects of curcumin against oxidative damage on skin cells *in vitro*: its implication for wound healing. Journal of Trauma. 2001; 51(5):927-931.
17. Reddy RC, Vatsala PG, Keshamouni VG, Padmanaban G, Rangarajan PN. Curcumin for malaria therapy. Biochemical and Biophysical Research Communications. 2005; 326:472-474.
18. Nabavi SF, Thiagarajan R, Rastrelli L, Daglia M, Sobarzo-Sanchez E, Alinezhad HN *et al*. Curcumin: a natural product for diabetes and its complications. Current Topics in Medicinal Chemistry. 2015; 15(23):2445-2455.
19. Zamrus SNH, Akhtar MN, Yeap SK, Quah CK, Loh W, Alitheen NB *et al*. Design, synthesis and cytotoxic effects of curcuminiods on He La, K562, MCF‐7 and MDA‐MB‐231 cancer cell lines. Chemistry Central Journal. 2018; 12:31.