



The Effect of Curcumin on the Invasion and Migration of Glioma Cells

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Authors' contributions

This work was carried out in collaboration among all authors. Author MZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MG managed the analyses of the study. Author TA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the effect of curcumin, from *Curcuma longa*, on the invasion of U87 glioma cell line spheres in 3D collagen model. Furthermore, this study investigated the anti-migration effects of curcumin on the migration of the same cell line in scratch assay.

Place and Duration of Study: Department of Pharmacology, School of Medicine, The University of Jordan, Amman, Jordan, between March 2019 and May 2019.

Methods: 3D invasion assay and 2D migration assays were used to fulfill these aims in addition to the Image J program that was used to analyze the area of invasion and the area of migration over the days of applying the assays.

Results: The results showed an inhibitory effect of curcumin in all samples tested on both the invasion and migration of U87 cell line.

Conclusion: This work adds more proofs on the importance of curcumin as anti-invasive and anti-migration agent and opens the door for more investigative studies.

Keywords: Cell lines; 3-D collagen model; scratch assay; glioma; curcumin.

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ABBREVIATIONS

- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
- European Collection of Cell Cultures ((ECACC),

1. INTRODUCTION

Research targeting tumor invasion have been enhanced in the last two decades. However, there has been no real advancements in investigating efficient methods to stop or hurdle metastatic growth. As a result mortality rates are increasing. Among the best strategies to fight metastases is to prevent its formation, however, these strategies have not yet entered routine clinical care, because of the lack of clinical validation studies. In this context, further studies are needed to explore potential preventive agents that could be helpful in preventing metastatic growth. The meaning of prevention could be explained on three levels. The first level includes preventing the cancer before it ever occurs by reducing exposure to risk factors. The second level includes reducing the effects and complications of cancer that already started. This is done by offering the needed treatment before the progression of cancer at the initiation stage of tumor. The third stage of prevention involves improving the ability to manage long term health problems associated with established tumors to limit its invasion of local or distant tissues, with hopeful increase in life expectancy.

This paper investigates the preventive effect of curcumin, a natural compound, against cancer invasion. It is always assumed that natural products would not have major harmful side effects and problems as compared with the available chemotherapeutic agents.

Curcuma longa, or turmeric, is the main source of curcumin which is known as the polyphenol Curcumin. Turmeric is well known and widely used oriental food spice. Recently, curcumin is well documented to have antioxidant effect in Indian and Chinese medicine [1] According to new studies, curcumin was found to have antiproliferative properties *in vitro* [2]. The interference with tumor cell cycle is supposed to be behind the curcumin properties of suppression of cancer cell growth [3]. Furthermore, curcumin has anti invasion properties due to its effect in regulation of growth factors and adhesion molecules [4]. This work,

aims to investigate the effect of curcumin on the invasion of U87 glioma cell lines in 3D spheroid invasion model. Furthermore, this work aims to investigate the effect curcumin on the migration of the same cell line in 2D scratch model. Finally, this work aims to compare the anti-invasion and anti-migration properties of U87 glioma cells in both the 3D invasion assay and the 2D migration assay.

2. MATERIALS AND METHODS

Curcumin is the generic name for (E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Diferuloylmethane, Pure curcumin powder from *Curcuma longa* was purchased from Sigma-Aldrich, USA.

2.1 The Cell Line and Reagents

The U87-MG Glioma cell line was purchased from the European Collection of Cell Cultures ((ECACC), Salisbury, Wiltshire, England). The cells were maintained in full RPMI 1640 medium and cultured at 37°C in a 5% CO₂ humidified atmosphere. Collagen I was purchased from Sigma-Aldrich (Poole, UK).

2.2 Methods

After treating the cells with different concentrations of curcumin, the cells were incubated with MTT at 37°C, 5% CO₂ for 4 hrs. MTT solution was removed and the optical density of the plates was read at 550 nm. The MTT Cytotoxicity assay was repeated three times. The MTT stock solution was prepared in 5 mg/ml concentrations and diluted to a final concentration of 0.5 mg/ml.

2.2.1 Collagen invasion assay

The U87 spheroids were prepared by the hanging drop method and seeded in 8-chamber cover glass (Nunc, Lab-TeK, Thermo Scientific) between two layers of Collagen I (pH 7.4). The assay was incubated at 37°C, 5% CO₂ for 7 days with daily images captured using inverted light microscopy. The spheroid invasion area was analyzed using ImageJ program.

2.2.2 Scratch assay

The scratch assay was done after the U87 cells reached 70 -80 % confluency. The curcumin was added after the cells were washed. The scratch

area was analyzed using Image J program which was used to analyze representative pictures of the scratch over the first 48 hours.

2.2.3 Statistical analysis

Student-test analysis was used to analyze the data. Results were considered statistically significant with p value = 0.05.

3. RESULTS

3.1 MTT Assay

MTT assay was done for the U87 cell after treatment with different concentrations of curcumin. The IC₅₀ was around 264.4 μ M. The concentrations for the 3D invasion assay and the

scratch assay were chosen to be less than IC₅₀ of curcumin.

3.2 Collagen Invasion Assay

The collagen invasion assay was performed on different concentrations of curcumin. The concentrations were chosen to be less than the IC₅₀ although one of the concentrations was chosen to be above the IC₅₀ for purposes of comparison. With a concentration round 6 μ M, there was relatively no effect of curcumin on the invasion of the U87 cells compared to the control. With concentration of 63.3 μ M, which is around one fourth of the IC₅₀, there was significant inhibition of the invasion of the U87 spheres in collagen (Figs. 1 and 2).

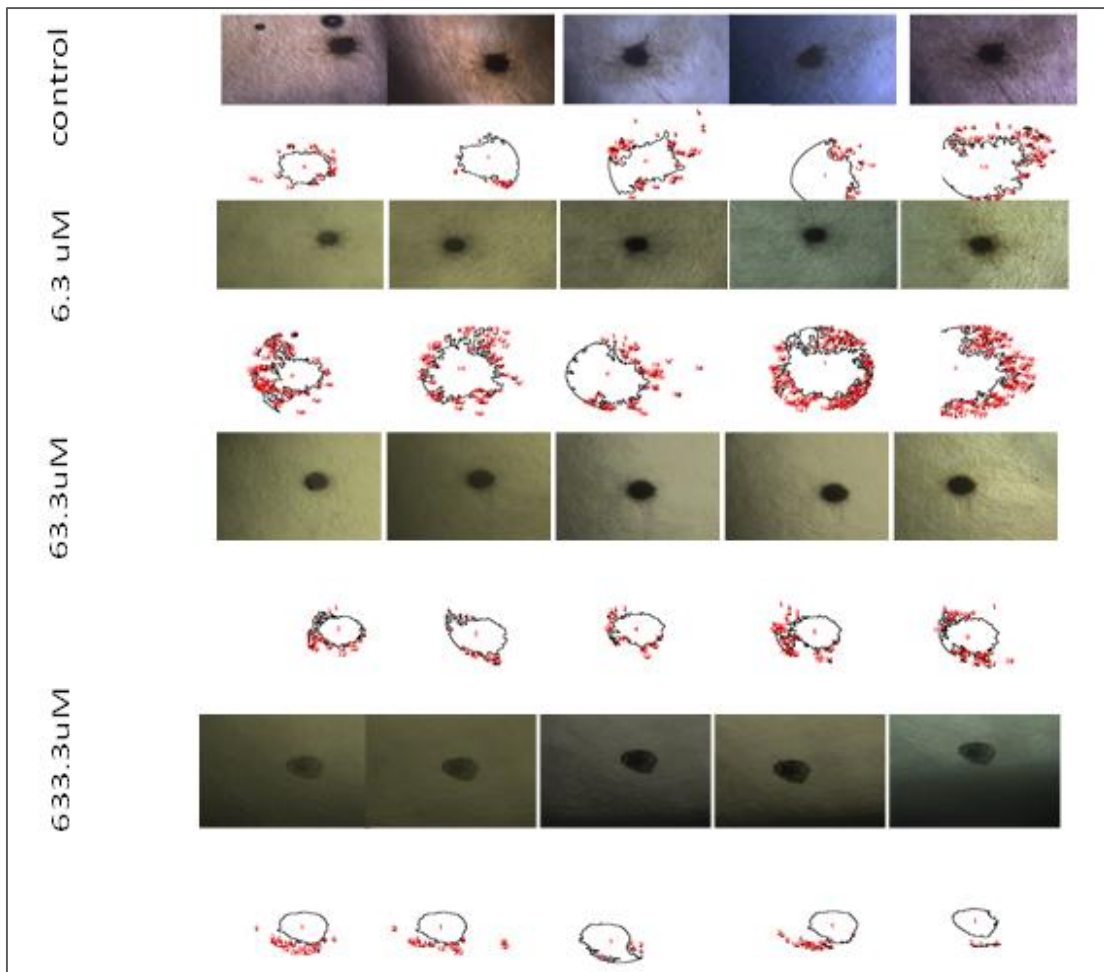


Fig. 1. The effect of different concentrations of curcumin on the invasion of U87 cells in 3D invasion assay

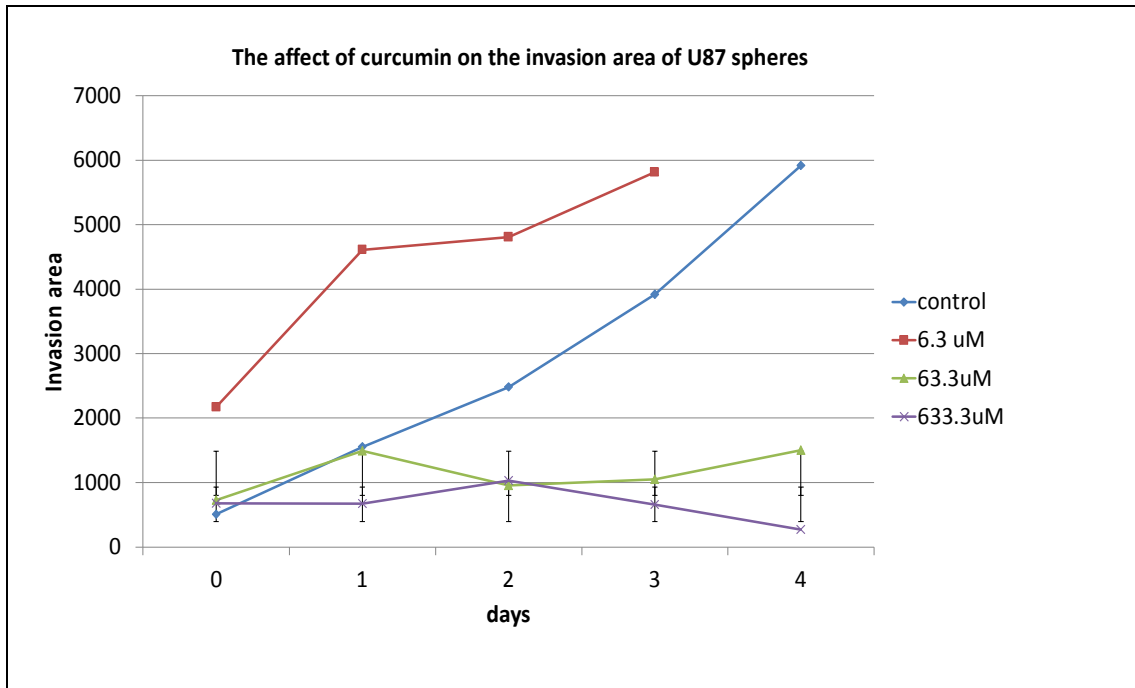


Fig. 2. Diagram showing the relation between the invasion area and time

3.3 Scratch Assay

Different concentrations of less than the IC50 were tested to determine the effect of curcumin on the migration of U87 cell in scratch assay. The concentration of 8.4 uM had comparable effect to the control. However, starting from the concentration of 35 uM, - a about one eighth of the IC50, a gradual increase in the inhibition of U87 cell in scratch assay was noticed (Figs. 3 and 4).

4. DISCUSSION

The 3D spherical invasion assay was used to investigate the effect of curcumin on the invasion of U87 cells. This is because this assay is considered as a representative model to the tumor *in vivo*. The 3D structure of the spheres is representative of 3D tumour mass in addition to the collagen layers which are representative of tumor microenvironment. Collagen makes a barrier layer between the drug and the tumor mass making this model more challenging in relation to drug delivery. Furthermore, putting the spheres in between tow layers of collagen guarantee the invasive behavior of the spheres and prevent the migration of the cells on the

surface plastic wells and this makes the movement of the cells more challenging and representative to the behavior of the tumors *in vivo*.

Curcumin has shown a significant inhibitory effect on the invasion and the migration of the U87 cells. This effect could be explained by many publications which showed that curcumin has an inhibitory effect on the expression of MMPs [5,6] in inflammatory diseases and many cell lines [7-10] The inhibitory effect of curcumin could be due to its effect in down- regulating the expression of Ap-1 and NF_B [11,12].

The spheres are composed of multilayers of cells and this make the 3D model challenging in relation to the delivery of the drug to the tumor cells compared to the models that are composed of cells in 2 D structures.

When comparing the 3D invasion assay and the 2D migration assay, the inhibitory effect of curcumin was seen in lower concentrations in 2D compared to the 3D model. These results explain why the 3 D model is more challenging and representative of *in vivo* conditions.

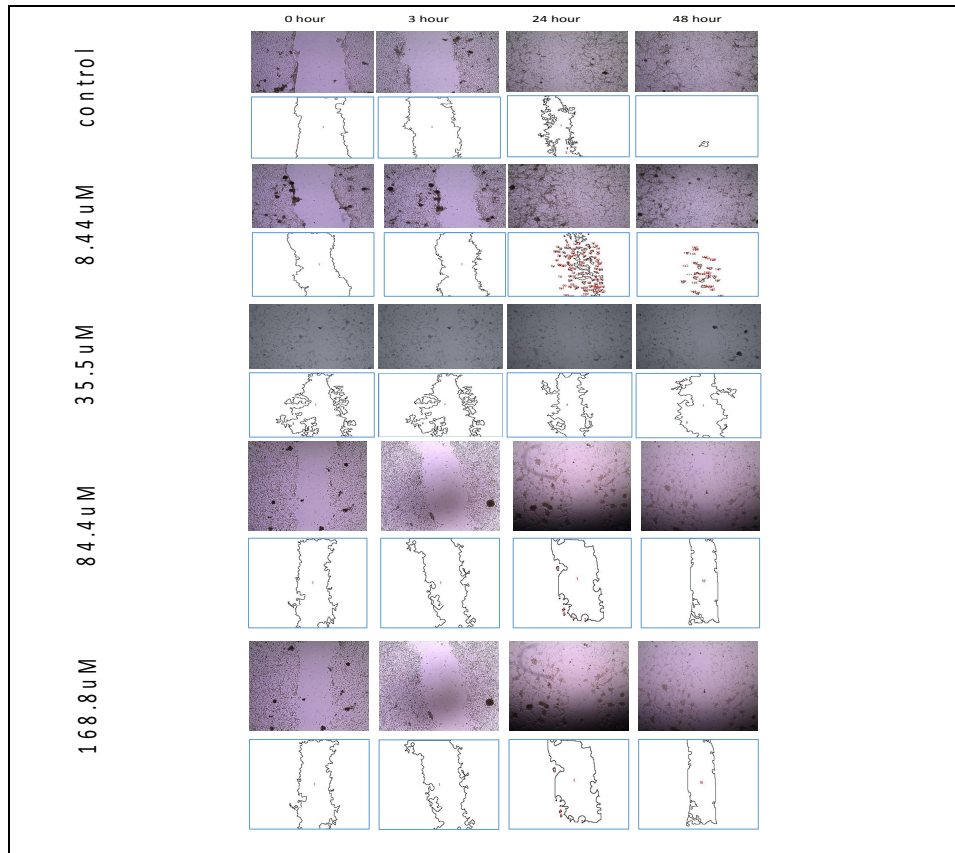


Fig. 3. The effect of different concentrations of curcumin on the migration area of the U87 cells in scratch assay

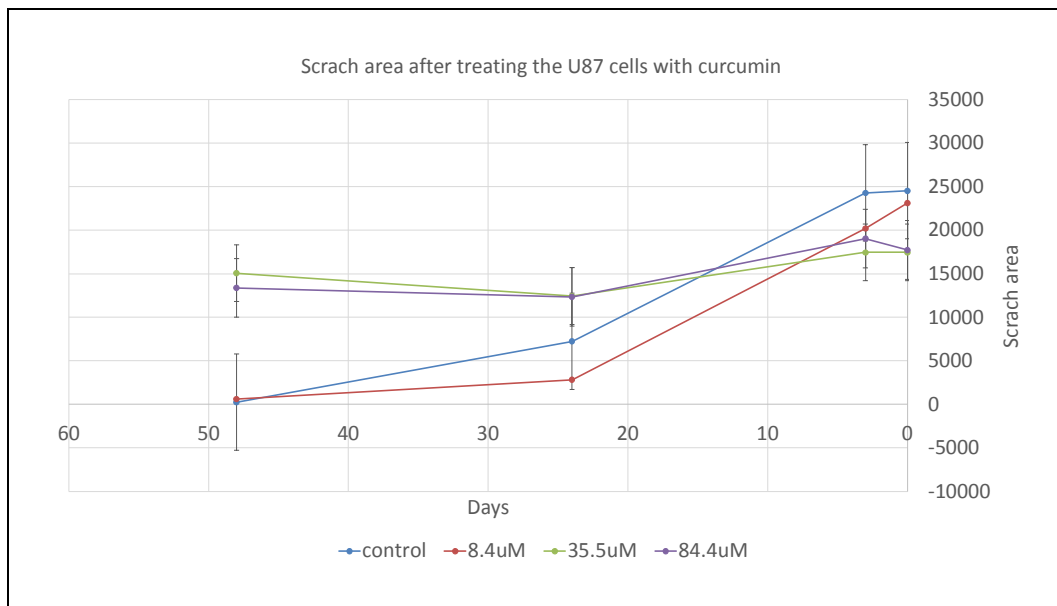


Fig. 4. Diagram shows the relation between the migration area and time in the scratch assay

5. CONCLUSION

Curcumin has shown an inhibitory effect on the invasion and migration of U87 glioma cells. The inhibitory effect of curcumin was gradual and started on concentrations much less than the IC 50. Further future studies are needed to investigate this effect of curcumin on the molecular level, and to have an idea about the mechanism of action of curcumin in inhibiting malignant cell growth.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chainani-Wu NJTJOA, C Medicine. Safety and anti-inflammatory activity of curcumin: a component of tumeric (*Curcuma longa*). 2003;9(1):161-168.
2. Mehta K, et al. Antiproliferative effect of curcumin (diferuloylmethane) against Human Breast Tumor Cell Lines. 1997;8(5):470-481.
3. Schwertheim S, et al. Curcumin induces G2/M arrest, apoptosis, NF-κB inhibition, and expression of differentiation genes in thyroid carcinoma cells. 2017;143(7):1143-1154.
4. Killian PH, et al. Curcumin inhibits prostate cancer metastasis *in vivo* by targeting the inflammatory cytokines CXCL1 and-2. 2012;33(12):2507-2519.
5. Swarnakar S, et al. Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. 2005;280(10):9409-9415.
6. Shakibaei M, et al. Suppression of NF-κB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: Implications for the treatment of osteoarthritis. 2007;73(9):1434-1445.
7. Mitra A, et al. Curcumin, a potential inhibitor of MMP-2 in human laryngeal squamous carcinoma cells HEP2. 2006;25(4).
8. Su CC, et al. Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor kappa B/p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions. 2006;26(2A):1281-1288.
9. Hong J, et al. The effects of curcumin on the invasiveness of prostate cancer *in vitro* and *in vivo*. 2006;9(2):147-152.
10. Lin LI, et al. Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion *in vitro* and suppresses matrix metalloproteinase-9 secretion. 1998;55(4):349-353.
11. Bachmeier B, et al. The chemopreventive polyphenol Curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. 2007;19(1-4):137-152.
12. Kim JM, et al. Curcumin suppresses the TPA-induced invasion through inhibition of PKCα-dependent MMP-expression in MCF-7 human breast cancer cells. 2012;19(12):1085-1092.

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