European Journal of Medicinal Plants

31(10): 38-43, 2020; Article no.EJMP.58225
ISSN: 2231-0894, NLM ID: 101583475

The Effect of Ginger 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 Supervised of the whole project, analyzed of data and wrote the manuscript. Author MG Helped in wrote the manuscript. Author TA conducted the cell line studies. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i1030279
Editor(s):
(1) Dr. Francisco Cruz-Sosa, Universidad Autónoma Metropolitana, Mexico.
(2) Marcello Iriti, University of Milan, Italy.
Reviewers:
(1) Praveen Kumar Uppala, Bhaskara Institute of Pharmacy, Andhra University, India.
(2) Govind Kumar Choudhary, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58225

Original Research Article

Received 18 April 2020
Accepted 23 June 2020
Published 03 July 2020

ABSTRACT

Background: This work studies the effect of different concentrations of soaked ginger on the ability of the U87 glioma cells to invade collagen in a three dimension (3 D) invasion model and compare it with its effect on the ability of the same cell line to migrate in two-dimension (2 D) scratch assay.
Methods: The hanging drop spheroids in 3D invasion assay were used to investigate the in invasion of the U87 cells. The 2D scratch assay was used to investigate the migration of the same cell line.
Results: Gradual effect of the soaked ginger was noticed on the inhibition of the invasion of U87 in collagen and on the inhibition of the migration of the same cell line in scratch assay.
Conclusion: The results in this article are promising and encourage further studies to investigate the effect of ginger active ingredients on tumour progression.

Keywords: Invasion; cancer; migration; assay.

ABBREVIATIONS

- *Three dimension (3 D)*
- *Two-dimension (2 D)*
- *Fifty percent inhibitory concentration (IC50)*
- *Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)*

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- *European Collection of Cell Cultures ((ECACC)*

1. INTRODUCTION

Ginger, or *Zingiber officinale Roscoe*, or *Zingiberaceae* is known as one of famous herbs in traditional medicine in addition to its use as flavoring agent. Inflammatory diseases have been dealt with by ginger for long time in traditional medicine [1]. The anti-inflammatory, antioxidant and anti-cancer properties of ginger are supposed to be due to the presence of active ingredients such as phenolic compounds including shogaol, gingerol, and paradol [2-16]. On molecular level, ovarian cancer cells treated by ginger extract have shown down-regulation of NF-κB-regulated gene products involved in cellular proliferation and angiogenesis, including IL-8, 20 and VEGF21 [17]. Furthermore, treating breast cancer cells with gingerol resulted in inhibiting cell proliferation due to presence of 10-gingerol which resulted in inhibiting mitogen induced Akt and p38MAPK activation and EGFR expression [18] The effect of ginger active ingredients such as gingerol and shogaol is extended to inhibit the invasion of breast cancer cells through down regulating MMP-2 and MMP-9 metalloproteinases which are known to be involved in inducing cancer cell invasion [19], [20].

In this work, the effect of ginger was investigated on the invasion of U87 glioma cells and on the migration of the same cell line. Tow assays were used to fulfill this aim. The first assay was the 3 D spheroid invasion assay, which was used to investigate the anti-invasive properties of ginger using concentrations less than the fifty percent inhibitory concentration (IC50) determined by dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The second assay was the scratch assay, which was used to investigate the anti-migration properties of different concentrations of soaked ginger selected to be less than the IC50.

2. MATERIALS AND METHODS

Ginger was purchased from the local market. Small pieces of the ginger root were soaked (*Zingiber officinale*) in the tissue culture water over-night. The soak was filtered by 0.45 um filters and aliquotted into 200ul aliquots and stored in -20 freezer until used.

2.1 The Cell Line and Reagents

The cell line used in the 3D model was U87-MG Glioma cell line purchased from the European Collection of Cell Cultures ((ECACC), Salisbury, Wiltshire, England). The U87 cells were maintained under standard culture conditions, at 37°C and 5% CO2 humidified atmosphere, as recommended by the ECACC.

2.2 MTT Assay

Different concentrations of ginger soak were used to treat the U87 cells then incubated with MTT for 4 hrs. The optical density of the plates was read at 550 nm and the test was repeated three times.

2.3 Collagen Invasion Assay

The hanging drop method was used to prepare the U87 spheroids which were seeded in Collagen I (Catalogue number C4243) in8-chamber cover glass (Nunc, Lab-TeK, Thermo Scientific, fisher/USA) at 37°C, 5% CO2 for 7 days. Daily images were captured and analyzed using Image J program.

2.4 Scratch Assay

After the U87 cells reached 70 -80 % confluence, the scratch assay was done. Different concentrations of the ginger soak were added, and many pictures were taken over the first 48 hours and analyzed using Image J program.

2.5 Statistical Analysis

The data was analyzed using Student t-test. Results were considered statistically significant with p values are between 0.05 and $p < 0.0$.

3. RESULTS

3.1 Scratch Assay

The different concentrations of ginger soak showed gradual ability to inhibit the migration of the U78 cells over the first 4 hours after treatment. The concentration of 0.66% (v/v) of ginger soak was comparable to the control in relation to the cell migration. However, starting from the concentration 1%, a significant gradual inhibition of migration was noticed. The concentrations 2% and 33.3% were comparable

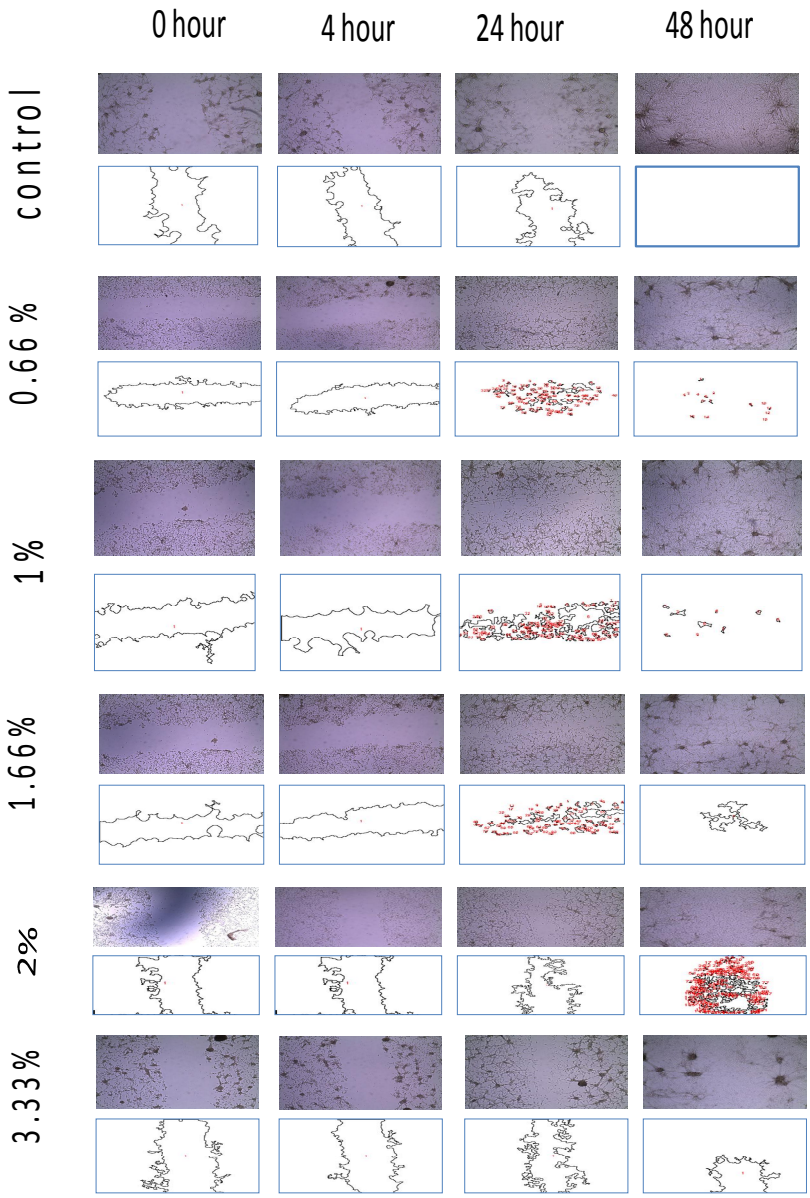

Fig. 1. Scratch assay after treating U87 cells with different concentrations of ginger soak indicating a gradual inhibition of the cell migration after 48 h of treatment

in their ability to inhibit the migration of the U87 cells in the scratch assay after 48 hours of treatment; (Figs. 1 and 2).

3.2 3D Invasion Assay

The effect of ginger soak on the invasion of U87 spheres in collagen was investigated through examining different concentrations in 3D invasion assay. Gradual inhibition of U87 spheres invasion was noticed after examining gradual concentrations of ginger soak; Figs. 3 and 4). In the first two concentrations, the effect of ginger was seen through having not continued increase in the size of the invasion after end of the 48 hours as seen in the control, Fig. 3 (B, C). The concentration of 25% (v/v) has shown a significant inhibition of the U87 spheres invasion, Fig. 3, D.

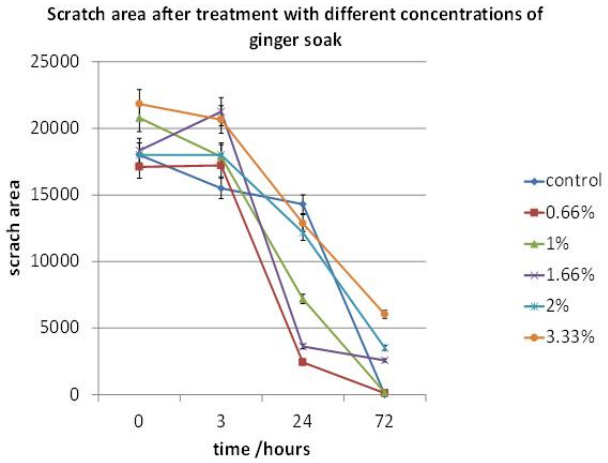

Fig. 2. Gradual inhibitory effect of ginger soak on the ability of the U87 cells to migrate in scratch assay after 48 hours of treatment

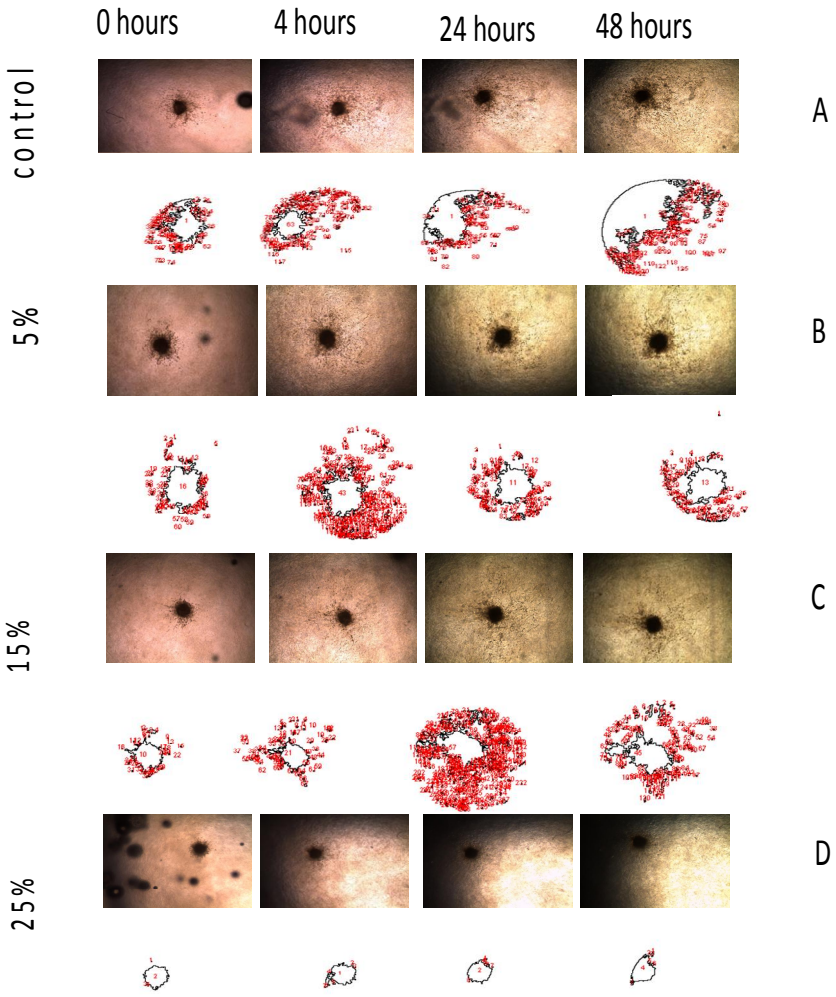

Fig. 3. Effect of different concentrations of ginger soak on the invasion of U87 spheres in collagen

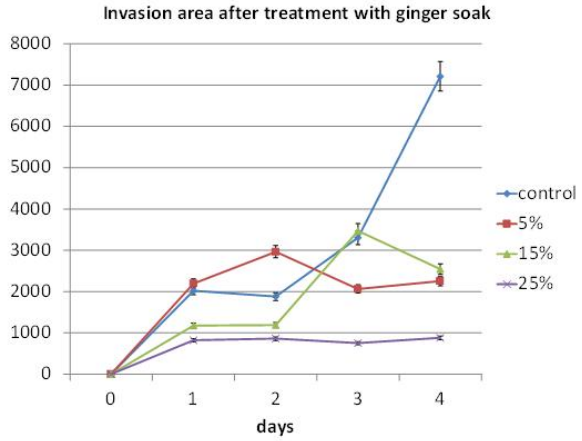

Fig. 4. Gradual inhibition of U87 invasion by different concentrations of ginger soak

4. DISCUSSION

The results show that the ginger soak had a gradual inhibition of U87 cells invasion and migration using the 3D invasion assay and 2 D scratch assay. The inhibitory effect of ginger was seen with lower concentrations in the 2 D migration assay compared to the concentrations used in the 3 D invasion assay. This effect could be due to different factors. First, the presence of collagen in the 3 D invasion assay exerts a challenging barrier between the drug and the cells compared to the 2 D scratch assay, where there is no barrier between the drug and the cells and the cells are in direct contact with the drug. Second, the presence of the cells in 3D spherical shape in the 3D invasion assay, render the delivery of the drug to cells more challenging compared to the 2D migration assay, where all the cells are in direct contact with the drug.

Regarding the 3 D invasion assay, the 25% ginger soak had shown significant inhibition of the invasion of the U87 spheres in 3 D invasion assay. The concentrations 5% and 15% had shown less inhibitory effect compared to 25%.

The results of this work coincide with previous works which show the antiinvasive and anti-cancer properties of ginger [2-17,19,20] indicating that ginger deserves further investigation for its anti-invasive and anti-migration properties.

5. CONCLUSION

This study shows a gradual inhibitory effect of the ginger soak on the invasion and migration of the U87. The ginger soak could inhibit the migration of U87 glioma cells on lower concentrations compared to those concentrations that inhibited the invasion in the 3D assay. This could be due to the more challenging properties of the 2D assay.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article [and its supplementary information files.

FUNDING

This work is funded by the dean of sientific research in the university of jordan.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58225