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The Effect of Pomegranate Juice on the Invasion and Migration of Glioma Cells

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ABSTRACT

Background: Cancer is considered as one of the fatal diseases in most countries. Despite the high medical care development, most cancers are resistant to treatment. Therefore, there is a continuous research for novel treatment methods. There have been increasing research interests in pomegranate as a result to its anticancerous effect, which is believed to be due to its high content of polyphenols. This work aims to study the effect of the pomegranate juice on the invasion of U87-MG spheres in 3D collagen model and on the migration of the same cells in 2D layer in scratch assay model. **Methods:** The 3 D collagen invasion assay and the 2 scrach assay was used to investigate the anti-invasive and anti-migration effects of pomegranate on the U87 cells. **Results:** Pomegranate juice has inhibited the invasion of U87- MG spheroids through the collagen in a dose and time-dependent manner. In addition, pomegranate juice showed more potent dose and time-dependent inhibition of migration of U87-MG cells in scratch assay. **Conclusion:** These results indicate that the 3D model was more challenging in evaluating the effect of pomegranate juice on the invasion of glioma cells

Keywords: Pomegranate, glioma cells, cancer, scratch assay

INTRODUCTION

The current problems associated with cancer chemotherapy resulted in a real shift toward natural alternatives for treatment of cancers (Bassiri-Jahromi, 2018a). Pomegranate is considered among those natural alternatives. Pomegranate (Punica granatum L.) is a round fruit with an outer hard shiny skin and an inner purple to reddish seeds that belongs to *Punicaceae* family. This fruit is widely consumed as raw seeds or as a juice by many people allover the world. Pomegranate has important medical history, and valuable medicinal properties (Bassiri-Jahromi, 2018a).

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Pomegranate is among the natural sources, which has shown to have an anti-proliferative and an anti-cancer effects against different cancer types such as breast, prostate, colon, and lung cancers (Longtin, 2003).

In addition to the anticancerous effect of pomegranate, it has been shown to have bioactive properties and an antioxidant activity (Sharma et al., 2017). Pomegranate is considered as a very important source of polyphenolics and tannin (Amakura et al., 2000a). Furthermore, the pomegranate peels contain active inhibitors, such as flavonoids and phenolics (Al-Zoreky, 2009). Practically, Pomegranate was found to be active against oxidative damage in diabetic rats (Longtin, 2003). Pomegranate has also shown to have an anti-invasive, anti-proliferative, anticancerous and anti metastatic effects in vitro and in vivo on different cancer cell line (Amakura et al., 2000b).

Although cancer as a whole is considered as a major human health problem, the tumor dissemination has

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a special interest, as it is the major cause of death for most kinds of cancers (Talmadge and Fidler, 2010).The difficulty in developing drugs that target controlling cancer is mainly due to tumor dissemination once it happens, in addition to the development of drug resistance by cancer cells (Glickman and Sawyers, 2012).

Tumor dissemination is a process that involves different definitions including invasion, migration, adhesion, metastasis and angiogenesis. Invasion is considered one of the most important steps in tumor dissemination process that includes alterations of many proteins (Barkan et al., 2010). Controlling the invasion and migration of the tumor is considered a key issue in the control of the whole dissemination process. This work aims to employ one of the important tumor invasion models, the 3D spheroid invasion model, to study the effect of the pomegranate fresh juice on the invasion of the U87 Glioma cells. This work also aims to investigate the effect of pomegranate fresh juice on the migration of the same cell line using the 2D scratch assay.

MATERIAL AND METHODS

Pomegranate Juice

Fresh Pomegranate juice was prepared from crushed pomegranate seeds without the peal. The freshly prepared juice was filtered using 0.45 filters and aliquotted then stored at -20C until used later.

The Cell Line

The source of the U87-MG Glioma cell line was the European Collection of Authenticated Cell Cultures ((ECACC), Salisbury, Wiltshire, England). The Cells were maintained under standard conditions. Briefly, the cells were maintained in full RPMI 1640 medium and cultured at 37°C in a 5% CO_2 humidified atmosphere.

Reagents and Solvents

Collagen I with catalogue number C4243 and all MTT assay reagents were purchased from Sigma-Aldrich (Poole, UK).

MTT Cytotoxicity Assay

The MTT cytotoxicity assay was done in triplicates. Briefly 5 mg/ml of MTT stock solution was prepared and diluted to a final concentration of 0.5mg/ml. The treated cells were incubated with MTT prepared solution at 37°C, 5% CO_2 for 4 hrs. After removing the MTT solution, the optical density of the plates was read at 550 nm.

Collagen Invasion Assay

Collagen I pH was corrected and prepared to a final pH 7.4. The U87 spheroids from hanging drops were seeded in 8-chamber cover glass (Nunc, Lab-TeK, Thermo scientific) between two layers of .the prepared collagen. After adding 200 µL of RPMI above the two layers of collagen they were incubated at 37°C, 5% CO_2 for 7 days. Daily images were captured for the spheroids for 7 days using inverted light microscopy at 10X objective lens. Image J program was used to analyze the spheroid invasion area.

Scratch Assay

After the cells were 70-80 % confluent the scratch assay was done. The cells were washed with media

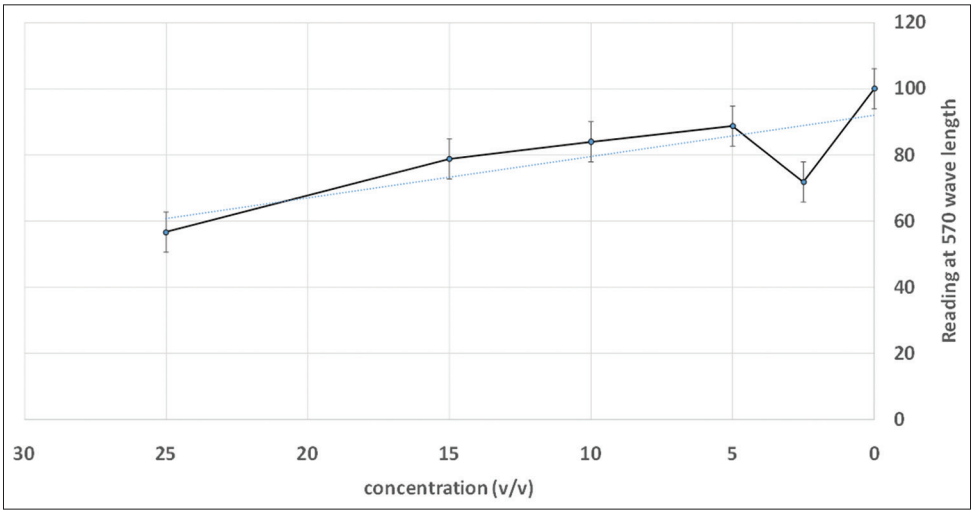

Figure 1: MTT assay done for pomegranate juice on U87 cell line

twice to remove unbound cells and the pomegranate juice with media were added. Representative pictures showing the area of the scratch were taken instantly, after 4 hours, 24 hours and after 48 hours. The pictures were analyzed by Image J program and analyzed as explained above.

Statistical Analysis

Statistical analysis of the data was performed using the t-test and the results were considered statistically significant if p values are less than 0.05.

RESULTS

MTT Assay

The MTT assay was repeated three times (Figure 1). The results showed that the IC50 of the used pomegranate juice was 18% (v/v). The concentrations used in the assays in this paper were selected to be less than this value.

3D Assay

The 3D invasion assay was repeated three times using three different concentrations, which are selected to be less than the IC50 as much as possible. The three different concentrations used were; 1.7% (v/v), 3.3%(v/v) and 5% (v/v).

The different concentrations of pomegranate juice showed a gradual inhibition of U87 spheres invasion in collagen as compared to the control (Figures 2 and 3). The results were statistically significant with p values between 0.05 and $p < 0.1$.

Scratch Assay

The scratch assay was repeated three time using different concentrations of the pomegranate juice. The results showed a gradual inhibition of the U87 cells migration. The effect was noticed on concentrations less than the concentrations used in the 3 D assay (Figures 4 and 5).

DISCUSSION

Pomegranate fruit, a popular constituent of healthy diet, is cultivated in many areas of the world particularly in the Mediterranean region (Lansky et al., 2000). The beneficial medical benefits of all parts of pomegranate fruit were known for thousands of years due to its high

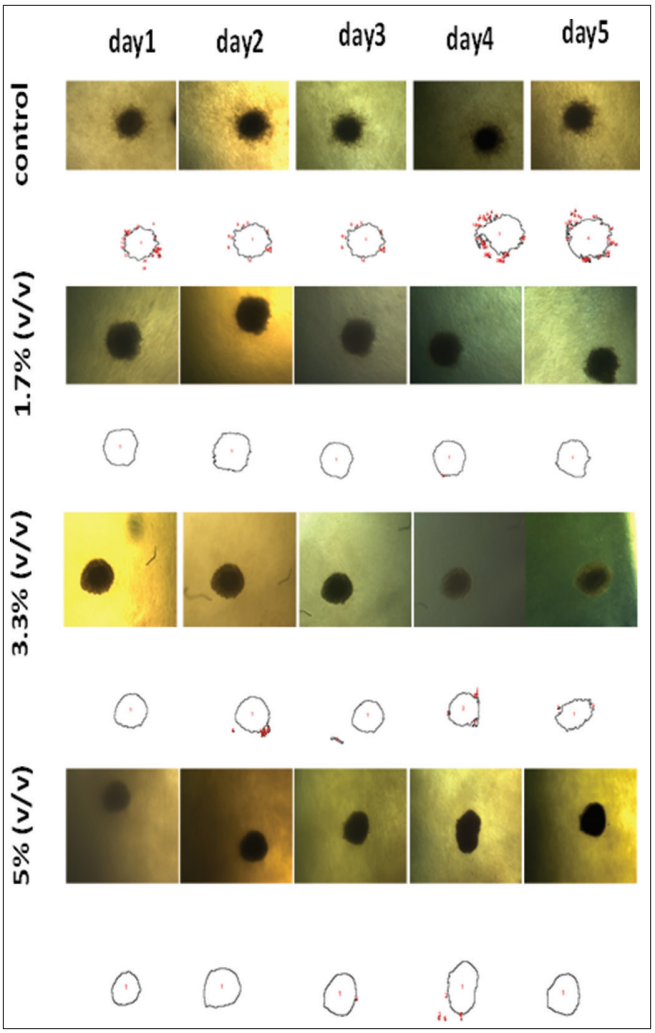

Figure 2: Gradual inhibition of U87 spheres invasion in collagen compared to the control

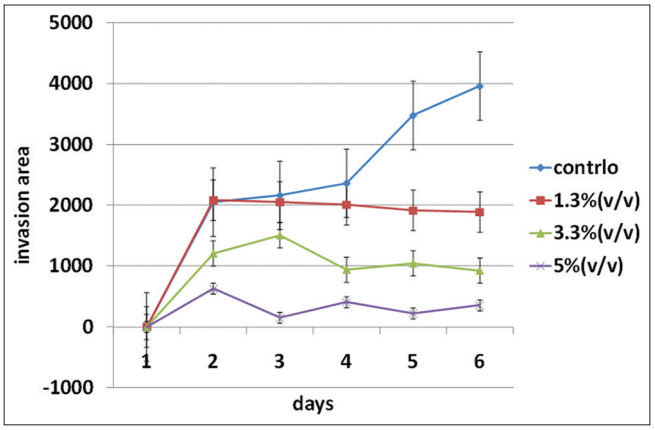

Figure 3: The effect of different concentrations of pomegranate juice on the invasion of U87 spheres

content of vitamin C and antioxidants. For example, it was used by many people in the management of diarrhea, sore throat, peptic ulcer, osteoarthritis, heart

diseases and diabetes mellitus (Lansky et al., 2000) (Ismail et al., 2012) (Colombo et al., 2013).

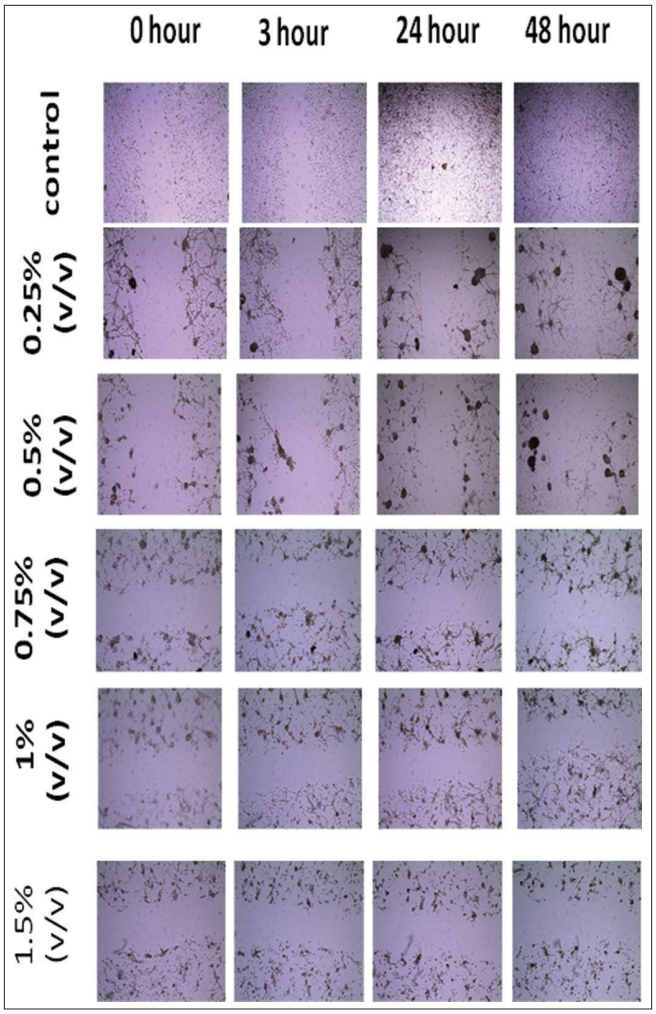

Figure 4: Gradual inhibition of U87 migration using different concentrations of pomegranate juice

Other traditional uses of pomegranate products have included hypertension, fertility aid, intestinal bacterial infection, intestinal inflammatory diseases as Crohn's disease, intestinal helminthes infestations and hemorrhage (Lansky et al., 2000).

In addition to the wide range of traditional clinical uses of pomegranate, it has been found that pomegranate juice, peel and oil have anticancerous activities, including interference with tumor cell proliferation, cell cycle, invasion and angiogenesis (Bassiri-Jahromi, 2018b). It is believed that the anticancerous effects to pomegranate are mainly attributed to its high anti-inflammatory and antioxidant properties (Lansky and Newman, 2007).

In the current study the effect of pomegranate juice on the invasion and migration of glioma cells was investigated. Gliomas are the most frequent invasive malignant tumors of the brain, and glioma cell lines are commonly used in research to assess anticancerous effect of drugs and their therapeutic application in terms of tumor growth, invasion, migration and angiogenesis (Remondelli and Renna, 2017) (Giakoumettis et al., 2018). Our results have shown gradual effect of pomegranate juice on the inhibition of U87 invasion and migration. The pomegranate juice has been more efficient in inhibiting the migration of the cells compared to its effect on the invasion. This effect is mostly because the 3D invasion assay is more challenging than the 2D migration assay.

The presence of collagen makes the invasion process more challenging and more able to mimic the in vivo situation. The 3D structure of the spheres makes the model able

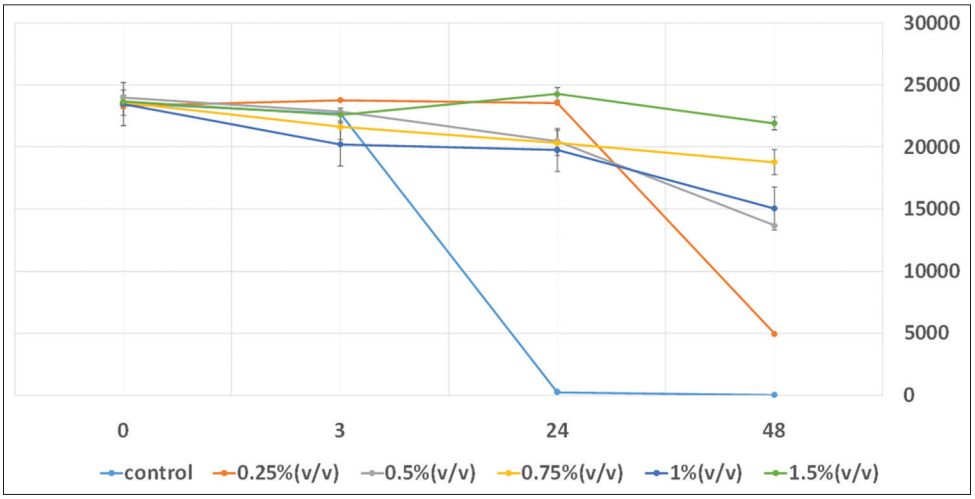

Figure 5: The effect of different concentrations of pomegranate juice on the inhibition of U87 cell migration

to mimic as much as possible the 3D structure of the in vivo tumors compared to the 2 D models in general.

CONCLUSION

The pomegranate juice is considered as promising agent in the field of Glioma treatment research that needs more investigation and analysis to characterize the active ingredients that are behind its anti-invasive and anti-migration effects.

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STATEMENT CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to authorship and/or publication of this manuscript.

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