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Original article

Effect of Quinoa Plant on Metastasis and Ion Channels of Rat Brain Cancer Glioma Cell Lines

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Abstract

In this study, the effects of the quinoa plant on the rat brain cancer glioma cell line were examined. Rat C6 glioma cell lines were cultivated in Dulbecco's Minimum Essential Medium (DMEM) appendage with HAMS F 12 (1:1) and 2% FBS. After proliferation, Quinoa plant was added into the cells and incubated at 37°C for 24 and 48 h in 5% CO2. The viability of the cells was identified by using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). IC50 concentration was determined using the statistic software SPSS (Probit analysis). The effect of the quinoa plant on the invasiveness of the C6 cells was analyzed by the wound test and the changes in the ion concentrations in the cells were determined with ICP-MS. As a result of the MTT test, the IC50 value of the quinoa plant was determined as 50 ppb. Wound test showed that use of quinoa plant (50 ppb) inhibited metastasis in the glioma cells while the cell proliferation in the control group was continued. Furthermore, calcium, sodium and potassium ions, which are regulators of cell cycle, were found in higher concentrations in that the untreated control cells than quinoa treated cells. As a result of this study; ICP-MS analysis showed that higher levels of calcium, sodium, and potassium ions were found in the untreated cells, whereas the application of the quinoa plant decreased these values. This change in ion channels was thought to be associated with the invasion of glioma cells, and it was determined that quinoa had significant anticancer effects.

Keywords: Quinoa, Cancer, Invasion, Glioma, Ion Channels.

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INTRODUCTION

Ion channels, which are among the basic building blocks of cells, have been found to have great importance in tumor growth and proliferation of cancerous cells by the researches made in recent years (Yıldırım, 2013). There are a number of genetic changes that affect the ion channels during the conversion of a healthy cell into a cancerous cell. Ion channels in cancer cells are connected to cell proliferation, apoptosis, migration, and metastasis. Metastasis, the most important life-threatening stage of cancer, involves a complicated process involving removal of cancerous tissue from the site of origin, deterioration of the basement membrane, entry into the circulatory system via blood or lymph, adherence to another tissue (adhesion), and formation of new blood vessels (angiogenesis) (Pancrazio et al., 1989; Marx et al., 1991). It is also known that Ca^{+2}, one of the regulators of the cell cycle, is increased in cancer cells and this increase is related to cell proliferation. Potassium channels are also exaggerated in cancer cells in high amounts (Pancrazio et al., 1989; Roger et al., 2006; Lu et al., 2008). Metastasis, the most important life-threatening stage of cancer, involves a complicated process involving removal of cancerous tissue from the site of origin, deterioration of the basement membrane, entry into the circulatory system via blood or lymph, adherence to another tissue (adhesion), and formation of new blood vessels (angiogenesis) (Mycielska et al., 2003; Hu et al., 2007; Erdogan et al., 2017). The relationship of various ion channels to metastasis has been examined and it has been found that ion channel alteration is not related to proliferation but to the invasion of metastatic cancers. In this study, the effects of quinoa plant on ion channels in rat brain cancer glioma cell line were examined.

Material and Methods

Plant Extract

After the quinoa plant was grown under suitable conditions, the leaves were removed and dried. After this procedure, 60 mg was weighed and 30 ml of distilled water was added. The stock solution was set to be 2 ppm. Then, serial dilutions were prepared from a stock solution and applied to the cells.

Culturing, Passaging and Freezing Cells

Cultivation, passaging and culture continuity of the cell lines used in our study were all done in sterile culture chamber and in the laminar cabinet (Safe Fast Elite (EN 12469 2000)). After the cells were obtained, 10 mg/ml streptomycin, 100 IU/ml penicillin, (MULTICELL (450-201-Z2) and 1% L-glutamine (MULTICELL (FBS-HI-IIA) (PANASONIC), incubated 95% humidity and 5% CO_2 at 37°C were added to EMEM:DMEM: HAMS F12 (MULTICELL) nutrient media flasks containing MULTICELL(609-065-E2). The adherence and other properties of cells were determined using an inverted microscope OLYMPUS) to ensure culture continuity. The cells growing in a healthy manner adhering to the bottom of the flask were passaged every three days. The trypsin-EDTA (MULTICELL 352-542-EL), which had been removed from the flask and removed to 37 °C) was added and the cells

were allowed to stand for 5 min from the flask bottom. The mixture of cell trypsin, which had been determined by the microscope, was taken into a 15 ml centrifuge tube. then centrifuged at 2500g for 2.30 min. After centrifugation, trypsin was removed and the remaining cells were mixed with nutrient and cultured in the new flask (NEST).

When the cells were frozen for reuse if necessary, the cells were centrifuged by trypsinization and then the cell culture mixture was added to contain 5% DMSO (MERCK 67-68-15) and stored at -150 °C (PANASONIC). When the frozen cells were to be reused, they were thawed at 37 °C, taken up into 15 ml centrifuge tubes and the dimethyl-sulphoxide (DMSO) removed by centrifugation (2.30 min). After addition of the new nutrient medium, cell culture flasks were inoculated and incubated under appropriate conditions and culture was continued.

Determination of Substance Concentration to be Applied to Cell Lines by MTT (3- (4,5-dimethylthiazol-2-yl) -2,5 diphenyltetrazolium bromide) Method

The MTT test was first performed with the intention of determining the doses of the quinoa plant extract to be applied to the cell and the application time.

In this method, the yellow colored MTT substance, which is reduced by mitochondrial enzymes of living cells, is transformed into purple water-insoluble crystals. DMSO is added to the crystals that come to the square and the absorbance is determined at 492 nm. The cell viabilities and IC_{50} values of 50% of the cells are calculated according to the absorbances determined.

Within the scope of this study, MTT method was applied to determine the activity of the quinoa plant to the glioma cell lines. To determine the IC_{50} value of the quinoa plant to be used in the study, 180 µL of the cells were inoculated into 96-well cell culture plates at 1×10^5 cells in each well and allowed to adhere to the plates by incubation for 24 hours. Following adhesion of the cells, the IC_{50} values were determined by applying the quinoa plant as 8 replicates.

ICP-MS assay

After IC_{50} value was determined, Cells were plated in 6 well plates and this IC_{50} value was applied to the glioma cells. After 24 hours of incubation, the application and control groups were allowed to incubate the cells overnight with nitric acid. 10-fold diluted in ICP-MS. and quantities of sodium, potassium, and calcium were determined.

Wound Healing Assay

Six well plates plated glioma cells were planted. It was incubated for 24 hours. After 24 hours, the medium was removed, and the wound was formed with a pipette. Washed twice with PBS. and the IC_{50} of quinoa plant was applied by adding two milliliters of media to each well. After 48 hours the medium was removed. Cells were washed with PBS and imaged under a microscope.

Results

Quinoa's anticancer effect

MTT assay was used to detect the effects of quinoa on cancer cells. Cells were incubated with increasing concentrations of quinoa depending on time. Significant differences were observed between the control and quinoa groups ($p < 0.05$). The effect of quinoa administration on cell viability was determined at different concentrations in the study. After 48 hours probit analysis was performed to calculate the IC_{50} value (50 ppb), which caused the death of 50% of the cells (Fig 1.)

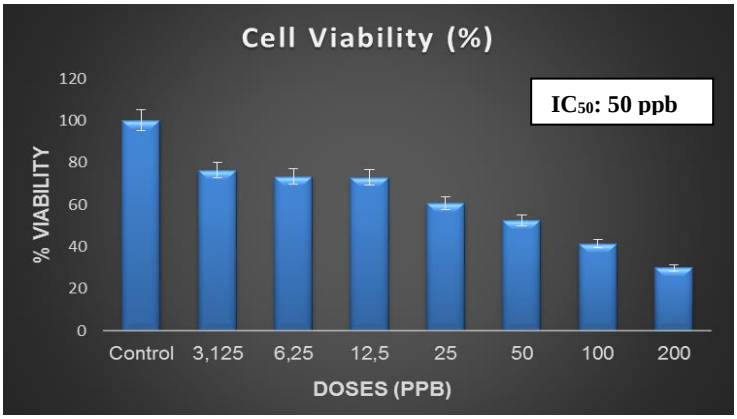

Figure 1. IC_{50} Value of C6 Glioma Cell Line Using MTT Test Results of Quinoa Application for 48 Hours (Probit Analysis)

Ion Change Results

In the analysis with ICP-MS device, it was found that the control group of Calcium, Sodium, and Potassium, which are regulators of the cell cycle, is higher than in the treated cells (Fig. 2.; Fig. 3.; Fig. 4.; Table 1).

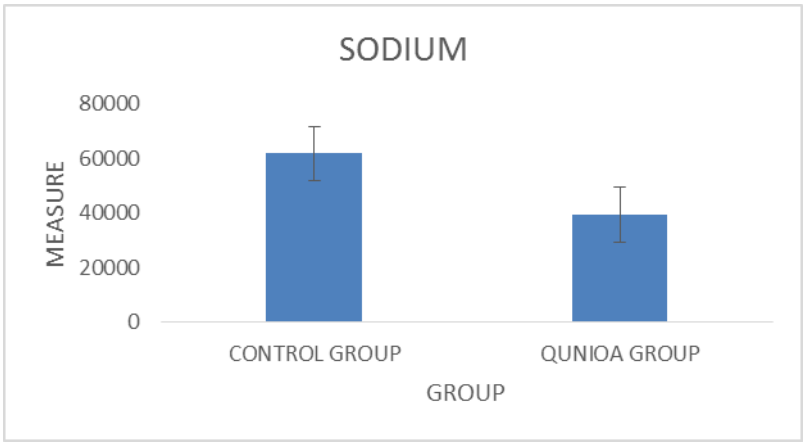

Figure 2. Sodium ion change to ICP-MS (Relative Coverage Increase n=3, Mean ± Standard Error (ANOVA-Duncan test, p≤0.05))

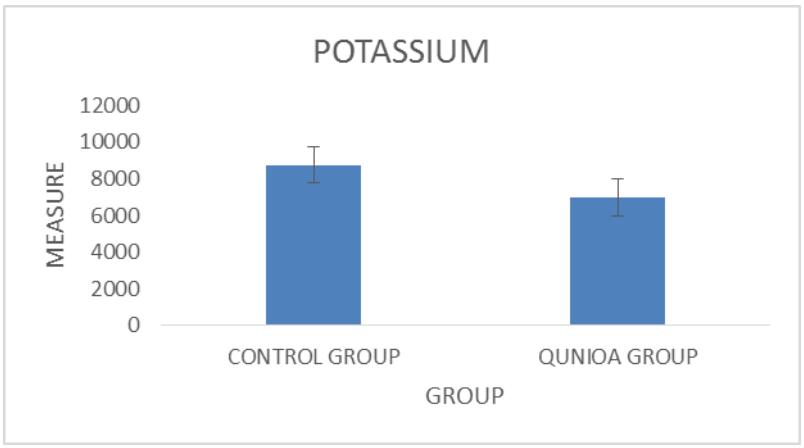

Figure 3. Potassium ion change to ICP-MS (Relative Coverage Increase n=3, Mean ± Standard Error (ANOVA-Duncan test, p≤0.05))

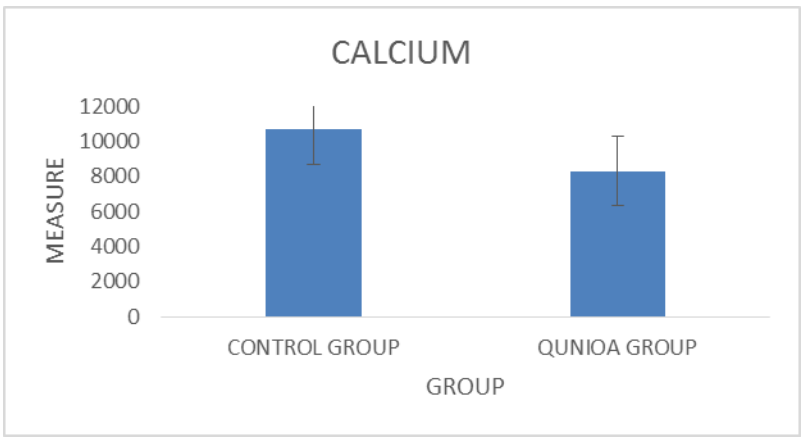

Figure 4. Calcium ion change to ICP-MS (Relative Coverage Increase n=3, Mean ± Standard Error (ANOVA-Duncan test, p≤0.05))

Table 1. Ion change analysis results with ICP-MS (n=3) ($p \leq 0.05$)

ppb	Control group	Quınoa group	Amount of decrease (fold)
Sodıum	61909.957	39386.326	1.572
Potassıum	8758.760	6989.472	1.253
Calcıum	10721.976	8321.098	1.289

Wound Healing Assay Results

At the end of 24 hours, the wound tissue in the control group was closed (98.8%) and the wound tissue formed in the quinoa treated group was not closed (77.7%). As a result of this experiment, quinoa plant was found to inhibit metastasis in glioma cancer cells (Fig. 5).

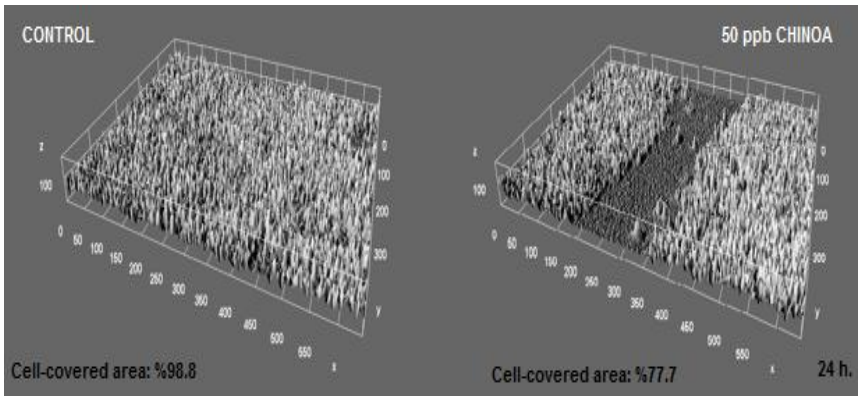

Figure 5. Wound Healing Assay Image

Discussion

There are many molecular mechanisms involved in the development of cancer. In our study, we examined the effect of these molecules on the metastatic process of cancer cells, the sodium, potassium, and calcium channels that are involved in the invasion and migration of cancer cells. And we searched for how this effect changed with the quinoa plant. It is known that the oncogenic development of most ion channels is necessary for the cellular processes involved. In addition to the uncontrolled proliferation of cancer cells, they digest the basal lamina before reaching the lymph and blood circulation and migrate to the tissues and form a second tumor when they reach the appropriate tissue. It is known that cell migration occurs with an increase in intracellular calcium. This event is controlled by potassium channels (Potier et al., 2006). There has been a lot of evidence in the last 10 years that sodium channels enhance the invasiveness of cancer cells in vitro conditions (Roger et al., 2006).

Sodium ducts are not excreted in the normal tissue or expressed only at a low level but they are abnormally expressed in the tumors. The level of these expressions is related to the formation of metastases in cancer cells. There has been a little study of the metastatic effect of sodium channels on cancer cells. Sodium channels are important in the electrogenic effects of neurons' skeletal muscle cells

and cardiac muscle cells. This role in the formation of diffusion is influential (Catterall, 2012). Over the last two decades, the expression of one or more of these channels in normal cells has been documented. Researchers have reported that sodium channels are more commonly associated with cell proliferation, differentiation and organization of cells during embryo development (Chopra et al., 2010; Kis-Toth et al., 2011; Bennet et al., 2013). Many studies described overexpression of sodium channels in cancer cells.

Since these cells are associated with increased cell invasiveness, they increase the risk of metastasis. They have also shown that they are not expressed in healthy cells or are at a low level (Campbell et al., 2013).

In one study, it has been shown that the activity of sodium channel is regulated variants of the domain VSG III in a Drosophila model, which differs in the voltage sensor S4 region. An increase was observed when channel activity was left uncontrolled or inhibited by phenytoin (Lin et al., 2012)

In the study of Han et al. (2007), pharmacological blockade of calcium-dependent potassium channels in human HeLa cervical and A2780 ovarian cancer cell lines induced cell cycle block and apoptosis in G1 phase together with increased p53 expression.

Fraser et al. (2005), *in vitro* assays on human breast carcinoma cells, showed a role in endocytosis of sodium channels, such as in prostate and breast cancer cases**.**

In 2002, Hara et al., demonstrated that heterologous overexpression of TRPM2 channel in rat insulinoma RIN-5F cells and U937 monocyte cell line increased H_2O_2 -induced apoptosis found that the antisense siRNA-mediated silencing suppressed cell death induced by H_2O_2 and TNF-a and the intracellular Ca^{+2} current to a significant extent.

As a result of the MTT test, the IC_{50} value of the quinoa plant was determined as 50 ppb. Wound test showed that use of quinoa plant (50 ppb) inhibited metastasis in the glioma cells while the cell proliferation in the control group was continued. Furthermore, calcium, sodium and potassium ions, which are regulators of cell cycle, were found in higher concentrations in that the untreated control cells than quinoa treated cells.

As a result of this study; ICP-MS analysis showed that higher levels of calcium, sodium, and potassium ions were found in the untreated cells, whereas the application of the quinoa plant decreased these values. This change in ion channels was thought to be associated with the invasion of glioma cells, and it was determined that quinoa had significant anticancer effects.

Conclusions

Qunioa is rich in protein, iron, potassium, many vitamins and minerals. The flavonoids found in the ingredients of quinoa act as antioxidants in the body and fight against free radicals, leading to the

formation of cancerous cells. Consuming quinoa regularly on this count can prevent both cancer cell formation and the spread of existing cancerous cells.

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