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GENETICS

The Extract of *Momordica charantia* Inhibits Cell Proliferation and Migration in U87G Cells

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Abstract—Glioblastoma multiforme is the most common brain cancer among central nervous system tumors. Although there are various treatment methods, these methods provide limited. Therefore, there is an urgent need for new more effective treatment strategies. This study, it was aimed to investigate the effect of *Momordica charantia* fruit extract on cell proliferation and migration using glioblastoma cells. The cells were treated with different concentrations of fruit extract and viability was measured by MTT analysis. The anti-migration effect of fruit extract on the cells was demonstrated by Scratch analysis. Also, the protein expression of Sparc and Src was investigated via the Western blot technique. Treatment of the fruit extract significantly resulted in decreased cell proliferation and the half-maximal inhibitory effect was detected as 700 μg/mL for 24 h. It inhibited cell migration ability and increased wound width on time-depending. Moreover, reductions in Sparc and Src protein expression levels were observed. The fruit extract exhibited a cytotoxic role by reducing proliferation and wound healing ability in glioblastoma cells depending on the concentration. Considering all the results, we proposed that inhibition of cell proliferation and migration is due to its effect on the expression of Src and Sparc proteins.

Keywords: glioblastoma, migration, *Momordica charantia*, Sparc, Src

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1. INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary brain tumor with the highest mortality rate in adults among all brain tumors (Tomar et al., 2020). The average life expectancy of GBM is 14 months (Lu et al., 2018) and more than 100000 patients die each year globally from this disease (Cenciarini et al., 2019). Due to inherent molecular and cellular heterogeneity (Cenciarini et al., 2019), it still maintains its place among deadly brain tumors by resisting treatment approaches such as surgical resection, radiation, and chemotherapy (Onay and Sengelen, 2019; Xing et al., 2020).

Momordica charantia, known as bitter melon, bitter gourd, balsam pear, and korela, is a popular plant belonging to the Cucurbitaceae family that is widely grown in tropical or subtropical regions (Fang et al., 2019) and is traditionally used in the treatment of disease in many societies (Yue et al., 2019). Since it contains various bioactive components in the structure of seeds, roots, leaves, and fruits (Yue et al., 2019), it has been used for various medical applications (Fang and Ng, 2011; Shodehinde et al., 2016; Bai et al., 2017; Ebrahim et al., 2020). Also, some studies show it causes anti-cancer activity by inhibiting RNA and cellular protein synthesis and DNA configuration (Puri et al., 2009).

Momordica charantia extract increased drug uptake into the cell by inhibiting P-glycoprotein activity in CaCo2 cells (Konishi et al., 2004) and multidrug-resistant KBV1 cells (Limtrakul et al., 2004). In a previous study, the leaf extract has been shown that inhibits invasion and migration in rat prostate cancer cells by causing matrix metalloproteinases (MMPs) inhibition (Pitchakarn et al., 2010). They also demonstrated that inhibits cell proliferation by inducing cell cycle arrest and apoptosis in human prostate cancer cells (Pitchakarn et al., 2011). Studies have also documented that bioactive components in the structure of *Momordica charantia* have anti-cancer potential against many types of cancer. A triterpene called TCD induced apoptosis by increasing caspase3, caspase7, and poly (ADP-ribose) polymerase (PARP) cleavage, and also increased tumor protein 53 (p53) and mitogen-activated protein kinase (MAPK) phosphorylation in breast cancer cells (Bai et al., 2016).

In this study, a water-soluble extract of this fruit was used to determine the cytotoxic effect and to investigate the mechanism underlying the inhibition of cell migration on human U87G glioblastoma cells. Although there are many studies in the literature examining the effect of its on various cancers, this study is the first to examine the effect of *Momordica charantia* fruit extract (MCFE).

2. MATERIALS AND METHODS

2.1. Cell Line and Reagents

U87G glioblastoma cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM), Sigma-Aldrich, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), Sigma-Aldrich, USA) and 1% penicillin-streptomycin (Sigma-Aldrich, USA) at 37°C in a humidified atmosphere of 5% CO_2 (Memmert CO_2 Incubator, INC153med, Germany).

The primary antibodies Src (c-Src/sc-8056) and Secreted protein acidic and cysteine-rich (Sparc/sc-73472) were purchased from Santa Cruz Biotechnology (Texas, USA). Beta-actin (β-actin/3700S), the anti-mouse secondary monoclonal antibody, and RIPA lysis buffer were obtained from Cell Signaling Technology (Boston, USA).

2.2. Preparation of Momordica charantia Fruit Extract

The powdered *Momordica charantia* fruit was weighed 50 g and after extracting with 150 mL of 96% ethanol for 6–8 h in the Soxhlet apparatus, the ethanol was removed. The evaporated sample was collected. The powdered extract obtained was dissolved with dH_2O and filtered through a 0.2 μm filter. The aqueous fruit extract was stored at –20°C.

2.3. Measurement of Cell Viability

The cytotoxic effect of MCFE on glioblastoma cells was measured by using MTT assay. Briefly, the cells were plated at 5×10^3 cells per well in a 96-well plate and treated at various concentration doses of the MCFE (100–1000 μg/mL) for 24 h. At this stage, only the medium was added to the negative control; the medium prepared with 20% DMSO was applied to the positive control, and the medium was prepared with dH_2O to the vehicle group. Then, MTT solution (5 mg/mL) was added and the cells were cultured under the same conditions for 4 h. The relative cell viability was measured by ELISA (Multiscan™ Microplate Photometer, Thermofisher Scientific, China) at 570 nm wavelength ($n = 3$).

The half-maximal inhibitory concentration (IC_{50}) dose and % cell viability was calculated by this formula;

$$Y = mx + C \text{ (Kaya and Eroğlu, 2021).}$$

(Y = inhibition, x = concentration, C = constant, m = coefficient.)

2.4. Cell Morphology Analysis

The glioblastoma cells were seeded into 6-well plates at a density of 5×10^4 cells. After incubation, MCFE was given to the cells at the concentration of its IC_{50} value. The cells were incubated for 24, 48, 72, and 96 h in the same conditions. After the culture medium was removed, the cells were washed with 1× PBS. The morphological structures of the cells were examined under an inverted microscope (Nikon Eclipse TS100).

2.5. Trypan Blue Dye Exclusion Analysis

A trypan blue dye exclusion assay was performed to examine the time-dependent effect of MCFE on glioblastoma cells proliferation. For this purpose, the cells of 5×10^4 were seeded into a 6-well plate and cultured. The cells were treated with MCFE at 24, 48, 72, and 96 h according to the IC_{50} value. After trypsinization, the cells were centrifuged and a culture medium and trypan blue dye (1 : 1) was added to the pellet. The cells were counted under an inverted microscope with a Neubauer hemocytometer ($n = 3$).

2.6. Scratch Assay

Scratch assay was performed to examine the effects of MCFE on lateral cell migration and cell-cell interaction. In short, the cells of 5×10^5 were seeded into a 6-well plate. A wound was opened on the adherent cells with help of a sterile pipette tip in the center of each well in a straight line. Cell residuals were washed with 1× PBS and removed. The cells were administered a dose of IC_{50} and their movements of its were observed at 24 and 48 h, their images were recorded with an inverted microscope ($n = 3$).

Wound closure percentages were calculated using this formula:

$$\frac{A_{0\,h} - A_{24\,h}}{A_{0\,h}} \times 100 \text{ and}$$

$$\frac{A_{0\,h} - A_{48\,h}}{A_{0\,h}} \times 100 \text{ (Akturk et al., 2016).}$$

($A_{0\,h}$: 0 h width; $A_{24\,h}$: 24 h wound width and $A_{48\,h}$: 48 h wound width.)

2.7. SDS-PAGE and Western Blot Analysis

Following the dosing of IC_{50} MCFE treatment for 24 h, the cells were centrifuged and added 1× RIPA buffer onto the pellet. After centrifugation, the supernatant was collected and protein concentration was

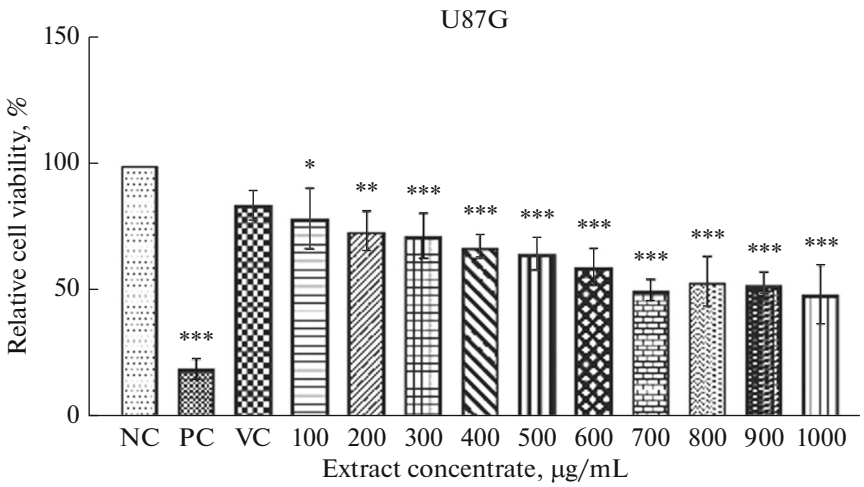

Fig. 1. The inhibitory effect of MCFE on the viability of U87G cells. All experiments were carried out in triplicate, and the data were expressed as mean ± SD for each experiment. * $P < 0.05$, *** $P < 0.001$ indicate significant P values.

measured by the Bradford method. The protein samples were denatured and loaded into 12% separating SDS-PAGE gel. It was then transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked and it was incubated with primer (1 : 500) and seconder (1 : 1000) antibodies at 4°C. Finally, it was incubated with ECL, and the data of the expression of target proteins by the G-box gel imaging system (Sygene, USA) were recorded and evaluated with the Image J program.

2.8. Statistical Analysis

All data were presented as means ± standard deviation (SD) and the statistical analyzes were performed by using a one-way analysis of variance (ANOVA) and an independent *t*-test. All data were analyzed using Microsoft Excel, Image J, and Graph Pad Prism 7.0 programs (GraphPad Software, La Jolla, USA). A value of (*** $p < 0.001$) was determined as significant.

3. RESULTS

3.1. Cell Viability Assay

The result presents that the cells with the MCFE significantly reduce in cell viability and this effect was due to the increased dose of its. Figure 1 shows the results of glioblastoma cells treated with MCFE for 24 h. The IC_{50} value of MCFE obtained as a result of MTT analysis is 700 μg/mL for the cells.

3.2. Trypan Blue Dye Exclusion Analysis

As seen in Fig. 2a, the IC_{50} dose of MCFE has been shown to cause an increased lethal effect at 16.6, 42.6, 79.3, and 91.6% respectively at 24, 48, 72, and 96 h, and the cell viability has decreased significantly depending on the time (*** $p < 0.001$).

3.3. Cell Morphology Analysis

The microscopic images of the morphological characteristics of the cells cultured for 24, 48, 72, and 96 h with the IC_{50} dose compared to the control group are shown in Fig. 2b. Time-dependent treatment with MCFE causes abnormal changes in cells such as an increase in nuclei density, a decrease in cell number, a reduction in cell size, and a rounded shape resulting from loss of cell extensions and adhesion properties.

3.4. Scratch Assay

The effects of MCFE on lateral cell migration and cell-cell interactions on glioblastoma cells were investigated by scratch assay. It was observed that the wound, which was recorded as 601.64 μm on the 0 h in the control group cells, was completely closed at the end of the 24 h. In the cells treated with MCFE, the width of the wound was measured as 497.44, 601.08, 733.04 μm at 0, 24, and 48 h, respectively. As shown in Figs. 3 and 4, it was determined that cell viability decreased, wound healing was inhibited, and wound width increased over time by inhibiting cell motility (*** $p < 0.001$).

3.5. Western Blot Analysis

The effect of Src and Sparc protein expression levels in glioblastoma cells treated with MCFE was confirmed using the Western Blot technique. While it completely inhibited Src expression in the cells compared to the control group at the end of 24 h of 700 μg/mL extract application; on the other hand, it greatly inhibited the Sparc expression (Fig. 5).

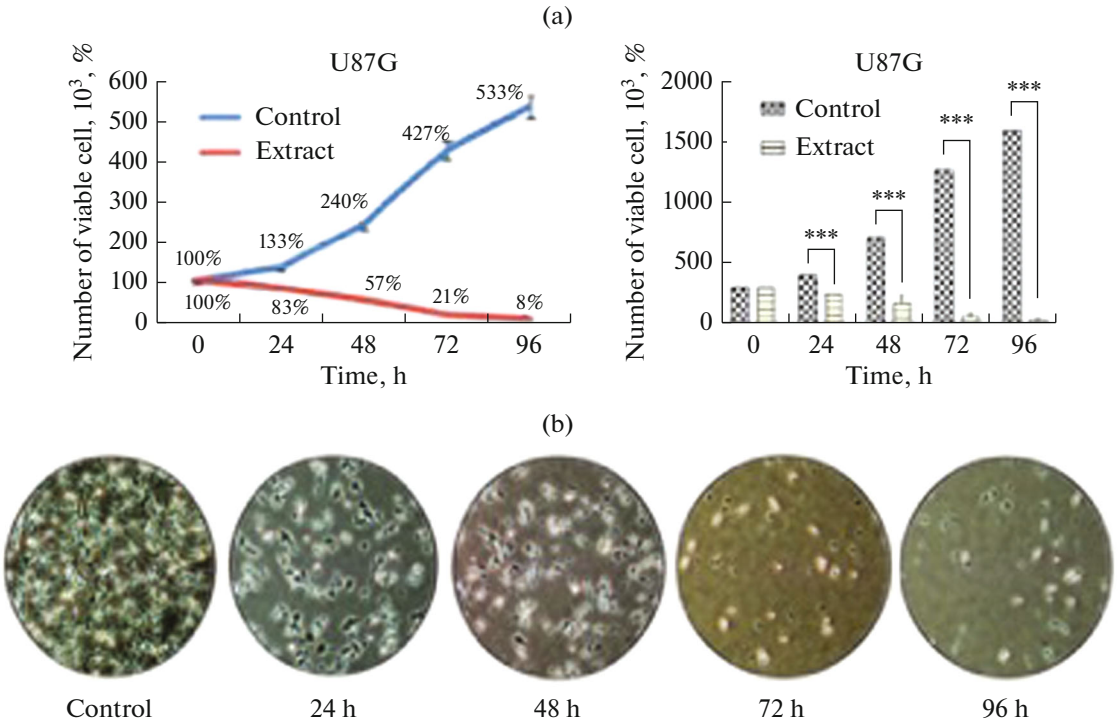

Fig. 2. (a) MCFE inhibits U87G cells proliferation time depending. Columns represent the average of three separate tests. *** $P < 0.001$ indicates significant P values. (b) Microscopic image of the effect of MCFE on cell morphology with respect to time (4× magnification-Nikon Eclipse TS100).

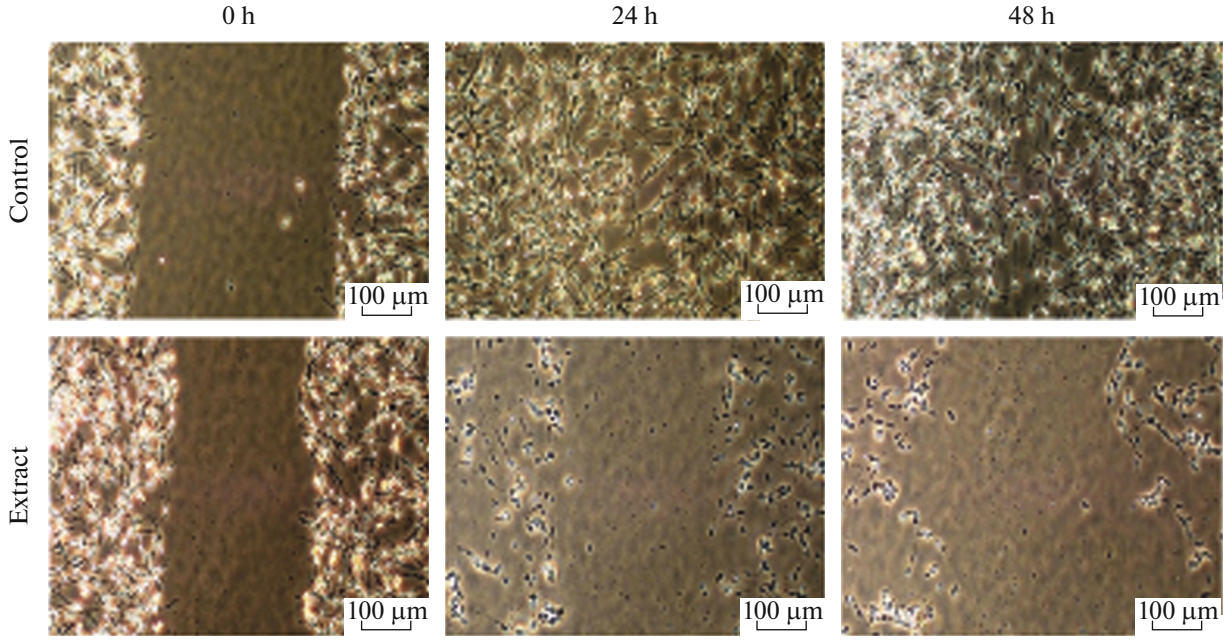

Fig. 3. Microscopic images of the wound widths of the control group and treated U87G cells with MCFE after 0, 24, and 48 h (4× magnification-Nikon Eclipse TS100).

4. DISCUSSION

Plants have a long history of use in cancer treatment and plant-derived compounds have been an important source of many clinically useful anticancer agents (Cragg and Newman, 2005). With the emergence of new techniques, barriers to natural compounds are being reduced and interest in the use of these natural ingredients in the pharmaceutical indus-

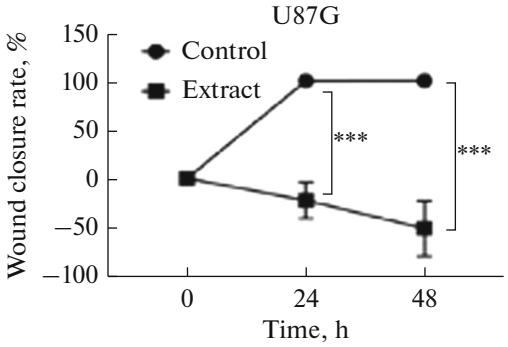

Fig. 4. The effect of MCFE on wound closure rate in U87G cells with respect to time. The experiments were carried out in triplicate and *** $P < 0.001$ indicates significant P values.

try is increasing. Based on the tests performed, it has been proven that plants with anti-cancer properties cause cytotoxic effects against cancer cells (Khan et al., 2019). Recent researches have focused on *Momordica charantia*, an equatorial herb popularly used in traditional medicine to treat many diseases, especially diabetes (Li et al., 2009; Fang and Ng, 2011; Manoharan et al., 2014). Previous studies with this herb have been confirmed to have anticancer properties against various cancer cells (Konishi et al., 2004; Pitchakarn et al., 2010; Bai et al., 2016; Fang et al., 2019).

As a result of the MTT experiment in our study, a decrease in the viability of glioblastoma cells was observed with increasing doses of MCFE and the IC_{50} value was determined as 700 μg/mL (*** $p < 0.001$). We think that MCFE has a cytotoxic effect in the cells, due to the triterpene-glycosides belonging to the Cucurbitaceae family such as charantagenin E, momordicaside K, goyaglycoside D and stigmata-7.25(27)-dien-3β-ol, especially charantagenin D in the study of Wang et al. (Wang et al., 2012), and also the α, β-momorcharin protein belonging to the RIP family in the extracted content in the study of Manoharan et al. (Manoharan et al., 2014). When we examine the literature, Pitchakarn et al. found the IC_{50} range of leaf extract to be 150–200 μg/mL in prostate cancer cells (Pitchakarn et al., 2010). In another study, Li et al. found the IC_{50} value of the extract they obtained from its leaves in nasopharyngeal, gastric adenocarcinoma, colon, and lung cancer cells, respectively 350, 300, 300, and 250 μg/mL (Li et al., 2012). The lethal effect of MCFE on glioblastoma cells in this study is in line with the literature. The reason for the difference in IC_{50} values compared with the literature is that different cancer cells give different responses to the extract in different environments, and also this may be due to the effect of the multiform structure, which contributes to the aggressive properties of glioblastoma cells.

In the present study, we determined that MCFE had an increasing lethal effect by 16.6, 42.6, 79.3, and 91.6% for 24, 48, 72, and 96 h (*** $p < 0.001$). Ray et al. found that 2% (v/v) extract application breast cancer cells reduced cell proliferation by 80% at 48 h (Ray et al., 2010). Ru et al. found that 2% (v/v) extract application on prostate cancer cells reduced cell proliferation by more than 90% at 96 h (Ru et al., 2011). It has been observed in our study and studies in the literature that it has an inhibitory effect on the proliferation of cancer cells. This indicates that MCFE is a potent cytotoxic agent for glioblastoma cells.

We defined that wound healing was prevented, and wound width increased depending on time by applying MCFE to glioblastoma cells (*** $p < 0.001$). Pitchakarn et. al. found that administration of 25–50 μg/mL

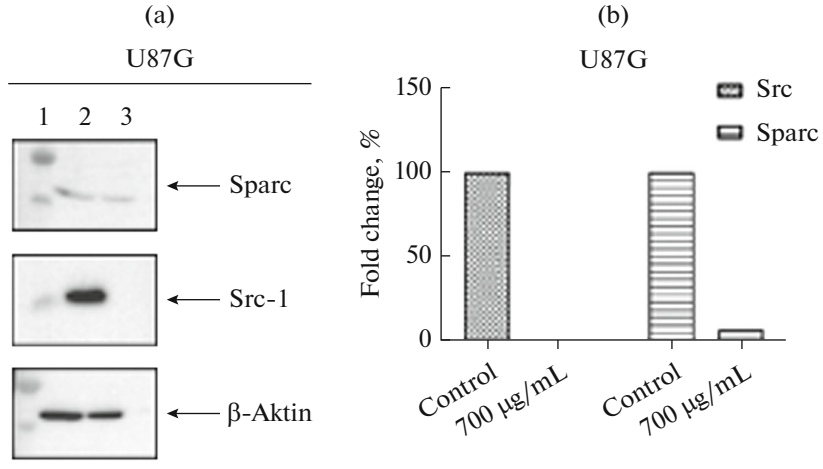

Fig. 5. (a) Effect of MCFE extract on expression levels of Sparc and Src proteins. Protein bands were quantified and normalized (1: marker; 2: control cell group without MCFE; 3: a group of cells treated with MCFE). (b) Fold changes in expression levels of proteins.

extract in prostate cancer cells inhibited the invasion ability of cells by 49 to 59% (Pitchakarn et al., 2010). Yung et al. found that in ovarian cancer cells, 5% (v/v) extract application reduced wound closure by 40%, and 10% (v/v) extract application reduced wound closure by 50% (Yung et al., 2016). In addition, Chipps et al., in their study on colon cancer, showed that cells were removed from the surface at the end of 24 h and there was by 70% reduction in total protein mass (Chipps et al., 2012). In this context, the reason for the increase in wound width with time is that MCFE may also have an inhibitory effect on proteins that provide adhesive properties to cancer cells. Therefore, the cells are thought to be easily removed from the surface by losing their adhesive properties.

SRC is an important proto-oncogene in the regulation of various physiological processes such as cell proliferation, cell movement, cell adhesion, angiogenesis, and survival (Calgani et al., 2016; Patel et al., 2016; Musumeci et al., 2017; Cirotti et al., 2020). It is overexpressed in glioblastoma cells and the inhibition of Src reduced the growth, viability, and migration of GBM cells in both in vivo and in vitro models (Musumeci et al., 2017). In this study, we investigated the effect of MCFE on the expression level of Src protein in glioblastoma cells. We showed that administration of 700 μg/mL MCFE in the cells completely inhibited Src protein expression at the end of 24 h compared to the control group. Hsu-HY et al. reported that leaf extract inhibited Src expression at concentrations of 0.6 and 1.25 mg/mL on CL1-0 and CL1-5 lung cancer cells, and accordingly it has been shown that it reduces cell invasion. They emphasized that this situation occurred due to the decrease in the expression level of focal adhesion kinase (FAK) protein, which is the molecular partner of Src in invasion (Hsu et al., 2012). It has been reported in the literature that the interactions of FAK and Src proteins activate phosphatidylinositol 3-kinase (PI3K) by forming key complexes and, cause loss of adhesion in the extracellular matrix (ECM) by causing degradation of focal adhesions and stress fibers (de Groot and Milano, 2009). It has also been shown to support invasive growth and malignant progression by stimulating the Src/STAT3/VEGF pathway, which causes stimulation of angiogenesis in the tumor microenvironment in case of hypoxia (Guarino, 2010). In the light of the literature information, the migration-reducing effect of MCFE in U87G cells in a scratch assay. It is thought that Src may be related to the key proteins involved in cell migration and invasion where it provides molecular communication on signaling pathways.

Sparc is a multifunctional protein in many biological processes such as cell differentiation, cell proliferation, migration, and modulating cellular interactions associated with ECM (Gagliano et al., 2006). It is highly expressed in human glioblastoma cells and is a glycoprotein that delays tumor growth by stimulating cell invasion both in vivo and in vitro (Kunigal et al., 2006). The application of 700 μg/mL MCFE in U87G cells inhibited the protein expression of Sparc at the end of 24 h compared to the control group. With our current study, we think that the contribution of Sparc to the migration of glioblastoma cells was negatively affected by the application of the extract. Kunigal et al. showed that increased SPARC expression on U87G cells had no effect on cell proliferation but caused a strong angiogenic response (Kunigal et al., 2006). Gagliano et al. examined the role of SPARC in reducing cell invasion by showing that MMP-2 and SPARC expression is downregulated by the effect of Resveratrol in glioblastoma cells (Gagliano et al., 2006). Our study suggests that the decrease in cell migration in the scratch assay and the increase in wound width depending on time by losing the adhesive properties of the cells may be due to the effect of the extract on Sparc expression.

CONCLUSIONS

MCFE showed an antitumor effect on U87G glioblastoma cells in this study; it has a cytotoxic effect on cancer cells and decreased cell proliferation dose-dependent. In addition, by inhibiting the expression levels of Src and Sparc proteins, which are involved in many biological processes including cell proliferation, migration, invasion, and angiogenesis, it caused the cells to lose their adhesive properties and showed a decreasing effect on cell migration. In the future, more pharmacological studies are needed to evaluate the in vivo tumor activities of *Momordica charantia* and to understand its underlying mechanisms of action.

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AUTHOR CONTRIBUTIONS

Conceptualization: E.K. and E.O.; Methodology: E.K. and E.O.; Validation: E.K. and E.O.; Formal analysis: E.K. and E.O.; References: E.K. and E.O.; Writing-original draft preparation: E.K.; Writing-review and editing: O.E.; Visualization: E.K. and E.O.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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