Ann Med Res

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Annals of Medical Research

journal page: www.annalsmedres.org

Potentilla fulgens root extract's effect on breast cancer (MCF-7) and osteosarcoma (U2OS) cells proliferation and migration

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ARTICLE INFO

Keywords:
Potentilla fulgens
Cancer
MCF-7
U2OS
Wound healing

Received: Apr 29, 2024
Accepted: Jun 12, 2024
Available Online: 28.06.2024

DOI:
10.5455/annalsmedres.2024.04.080

Abstract

Aim: This study evaluates the cytotoxic effect of *Potentilla fulgens* root extract on MCF-7, U2OS, and RPE-1 cell lines and investigates its impact on cell migration.

Materials and Methods: The effect of *Potentilla fulgens* root extract on the cell viability of MCF-7, U2OS, and RPE-1 cells was analyzed using an MTT assay. The effect of the compounds on cell migration was evaluated at 24 and 48 hours using the wound healing assay. A wound-healing assay was used to measure the metastatic ability of cells *in vitro*.

Results: MTT assay showed that PRE had a cytotoxic effect on all three cell lines, depending on dose and time, and this effect was statistically significant. The wound healing assay results showed that PRE slowed the migration of live MCF-7 and U2OS cancer cells, and these effects increased over time.

Conclusion: As a result, this study indicates that PRE may be very useful in treating human osteosarcoma and breast cancer.

Introduction

Cancer, as a multi-stage disease, is one of the significant health problems to be tackled; it can affect different tissues and organs in the body [1-3]. According to the World Health Organization (WHO), cancers are responsible for 63% of deaths worldwide [2, 4]. The most important reason for this high incidence is that it cannot be diagnosed early [4]. Along with all these, it is estimated that there will be an increase of 47% in cancer cases globally in 2040 compared to 2020 [1-3, 5].

It is essential for many physiological processes, including cell migration, embryonic development, wound repair, tumor invasion, and neoangiogenesis, and is also an integral part of metastasis [6-9]. Metastasis is the leading cause of cancer-related deaths, accounting for over 90% of all cancer deaths. With early diagnosis and prevention of cancer growth, the cancer survival rate has increased significantly over the years. However, when cancers metastasize and spread beyond the primary site, they are often untreatable and fatal [10-14]. Osteosarcoma is a highly malignant bone tumor that affects children and adolescents. Survival rates are poor, especially in patients with lung metastases. Although chemotherapy and surgical techniques have reduced mortality in osteosarcoma, the 5-year survival rate remains at only 20% for patients with metastases [15, 16].

While as breast cancer causes the most cancer deaths among women. Death from breast cancer results from metastasis of transformed cells to other parts of the body. Therefore, finding methods to prevent cell migration in breast cancer will provide significant progress in the treatment of this disease [17]. Recently, there has been an increasing interest in using traditional medicinal plants as potential agents for cancer prevention and treatment [18]. One of these agents is the *Potentilla fulgens* plant, used for various diseases among the people in India. This plant, which grows in high-altitude regions of India, is an annual plant with yellow flowers and high anticancer, antitumor, and antioxidant activity [18-21]. Moreover, modern pharmacological studies have shown that extracts of *Potentilla*

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fulgens, which have been used traditionally as a medicinal plant for centuries, do not have toxic effects on humans [18, 22, 23].

This study aimed to determine whether *Potentilla fulgens* root extract (PRE), which is shown as one of the natural alternatives to chemotherapeutic drugs used in cancer treatment, has a cytotoxic effect on MCF-7 (Human Breast Carcinoma), U2OS (Osteosarcoma), RPE-1 (Retinal Pigment Epithelium) and to investigate its effects on cell migration.

Materials and Methods

Preparation of Potentilla fulgens root extract (PRE)

PRE was supplied as a dried powder from Xi'an Yuensun Biological Technology Company (China). It was dissolved in 2% ethanol, kept in a boiling water bath for 10 minutes, and cooled. The cooled solution was centrifuged in glass tubes at 2000 rpm for 10 minutes. The resulting supernatant was left at 4°C for the study [24-26].

Cell culture and Cytotoxic activities of PRE

Cell viability tests were performed on cancer cell lines MCF-7 and U2OS, and the healthy RPE-1 cell line. The cell lines were obtained from the American Type Culture Collection (ATCC) and tested at Dicle University's Faculty of Veterinary Medicine Cell Culture Laboratory.

Cell line was grown in DMEM (Gibco 41965039, England), supplemented with 10% FBS, 2 mM L-glutamine, and 100 units/ml penicillin/streptomycin in a T75 flask. The culture was then incubated at 37°C in a 5% CO_2 environment.

When the cells reached 80-90% confluency, they were removed from the flasks, and the number of cells was determined using the hemocytometric method. The cells with known numbers were then seeded in 96-well plates with $5x10^3$ cells in three replicates in two plates (for two separate time applications, 24 and 48 hours) with 90 µl of medium in each well. The plates were left for 24 hours to allow the cells to adhere.

The next day, PRE, which we had prepared at various concentrations (Final doses: 400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml), was applied to the seeded plates. In the control group, the cells were treated with 2% ethanol.

An MTT test was conducted at 24 and 48 hours after the application to determine changes in cell viability. 10 µl of a prepared MTT (5 mg/ml) solution was added to each well, and the cells were incubated for 3 hours at 37°C in a humid environment containing 5% CO_2. After 3 hours, the medium was removed, and 100 µl of DMSO was added to each well. The plates were left on the shaker for 20 minutes, and then the optical density (OD) values in the wells were determined with a UV/Vis Spectrophotometer (Multi Scan Go, Thermo) [27].

The absorbance values obtained from the control wells were accepted as 100% live cell values. The viability percentage was calculated by comparing the absorbance values obtained from the wells where PRE was applied to the control absorbance value. MTT trials were repeated 3 times on different days.

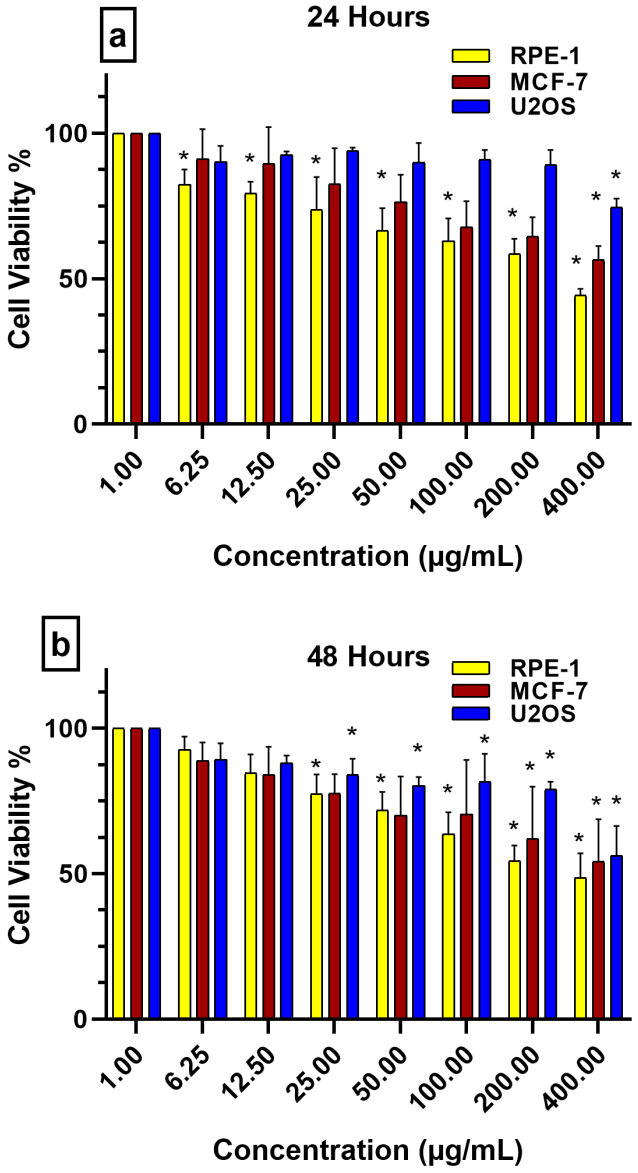

Figure 1. RPE-1, MCF-7, and U2OS cells were treated with different concentrations of PRE (400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml) for (A,a) 24 h. and (B,b) 48 h. MTT assay was performed to detect the viability of RPE-1, MCF-7, and U2OS cells. RPE-1, MCF-7 and U2OS cell viability were suppressed by PRE in a time and dose-dependent manner. The experiments were repeated three times independently, and all treatments were carried out in triplicates. The data were presented as mean ± standard deviation, and statistical significance was considered at *P $<$ 0.05 compared to their respective control groups.

The formula used to calculate Cell Viability is as follows:

Cell Viability (%) = OD treatment / OD control × 100% [28, 29].

Cell migration assay

The Cell Migration Experiment was designed to examine the PRE application's effect on cancer cells' metastatic be-

havior. The cell lines used in this study were transplanted into 6-well plates. After the transplanted cells became 80% confluent, a wound area was created by drawing a straight line from 12 o'clock to 6 o'clock with the help of a 200 µl sterile pipette tip.

After rinsing the wells twice with PBS to eliminate dead cell remnants, a treatment medium containing 200 µg/ml PRE was applied to the appropriate groups. The control groups were only given a plain medium. To assess the cells' ability to migrate towards the wound area and the rate at which the wound area closed, images were captured from all groups using an inverted microscope at 24 and 48 hours. The images were then comparatively evaluated [30].

The formula used to calculate the percentage of wound closure is as follows:

Wound Closure (%) = $(A_0 - A_1) / A_0 * 100$ [31].

A_0= Initial Scratch Area

A_1= t-Instantly Measured Scratch Area.

Statistical analysis

The obtained values of inhibitor concentration IC_{50} and $logIC_{50}$ values for PRE were calculated with the Graph-Pad Prism 8 program. The image-j program was used to analyze wound healing, and wound healing was expressed as a %. IBM SPSS 21.0 was used to analyze the study data at the $p<0.05$ level of statistical significance. Analysis among groups involved one-way analysis of variance, followed by Tukey's test [27, 32, 33].

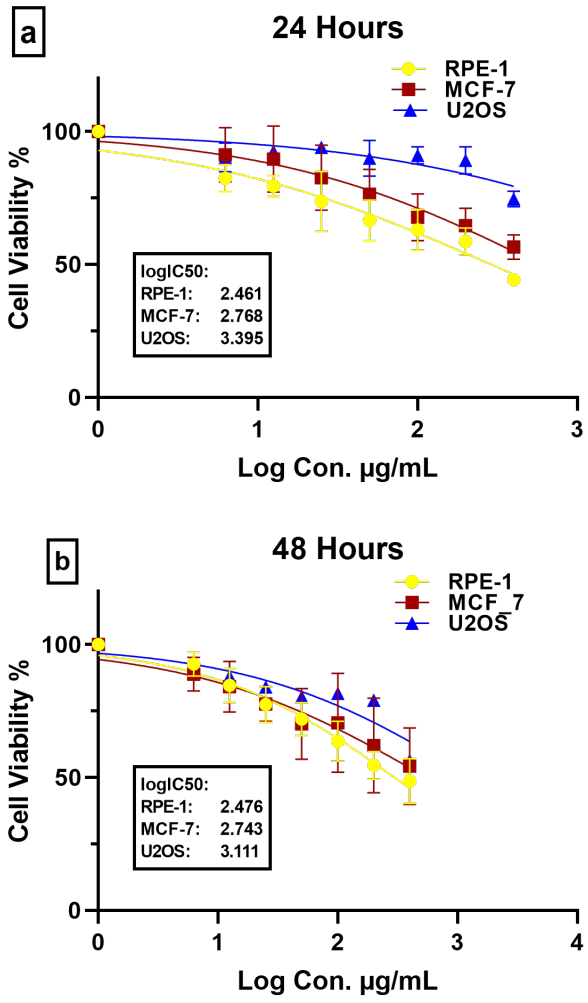

Figure 2. $LogIC_{50}$ of PRE (a:24 and b: 48. hours).

Results

Effects of PRE on cell viability

The MTT method examined the cytotoxic effects of PRE on MCF-7, U2OS cancer cells, and healthy RPE-1 cell lines. Table 1, Figures 1, and 2 give data on PRE's actions on MCF-7, U2OS, and RPE-1 cell lines based on the findings of the MTT assay.

When the outcomes were examined, it was determined that PRE had a cytotoxic effect in all three cell lines, depending on dose and time, and this effect was statistically significant. 56.56% and 54.29% cytotoxic effects were observed at the highest concentration applied to the MCF-7 cell line at 24 and 48 hours, respectively. This rate was 74.60% and 56.16% for the U2OS cell line. In the RPE-1 cell line, which is our health cell line, this rate was found to be 44.28 and 48.69%, respectively (Table 1). After 24 and 48 hours of application, $logIC_{50}$ values in RPE-1, MCF-7, and U2OS cells were 2.461, 2.476, respectively; 2.768, 2.743; It was detected as 3.395 and 3.111 µg/ml (Figure 2).

Cell migration assay

Figures 3 and 4 present the effects of PRE, which we applied to the cell lines used in the study, on cell migration. As seen in Figures 3 and 4, all cells to which PRE was applied showed an anti-migratory effect at the end of the 24^{th} and 48^{th} hours.

When Figure 4 is examined, it is seen that the scratch closure rate of MCF-7 at the end of 24 hours was 38.57% in the control group and 15.66% in the treatment group. At the end of 48 hours, although the scratch was almost completely closed with 94.93% in the control group, this rate decreased further compared to 24 hours and was found to be 3.36% in the treatment group. The scratch closure rate was found to be statistically significant at both times.

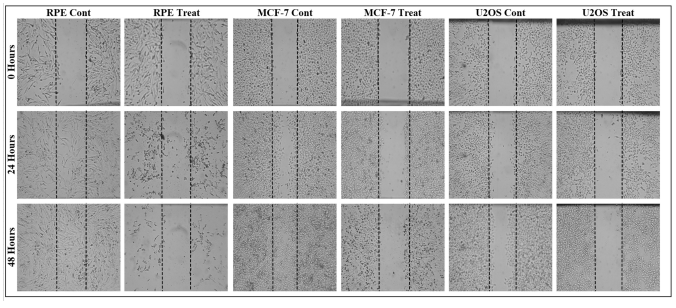

Figure 3. PRE has an antimigration effect on healthy and cancer cell lines. Through a wound-healing assay, we investigated the impact of PRE on the mobility of RPE-1, MCF-7, and U2OS cell lines. The cells were incubated for 48 hours in the presence or absence of PRE. At 24 and 48 hours, we used an inverted microscope to observe the cells migrating into the space. The gap's borders were highlighted with black lines [28].

Table 1. Percentage values of mtt assay results in RPE-1, MCF-7 and U2OS cell lines (A: 24 Hours, B: 48 Hours).

A	Cytotoxic effects of PRE on cell lines (n=3, $\overline{X}$± S$\overline{x}$24 Hours)						
	6.25 µg/mL	12.5 µg/mL	25.00 µg/mL	50.00 µg/mL	100.00 µg/mL	200.00 µg/mL	400.00 µg/mL
RPE-1	82.44±05.14	79.42±03.96	73.75±11.24	66.54±07.71	63.00±07.77	58.62±05.14	44.28±02.23
MCF-7	91.25±10.16	89.66±12.45	82.62±12.26	76.41±09.31	67.78±08.82	64.61±07.70	56.56±08.32
U2OS	90.29±05.39	92.57±01.19	94.06±00.99	89.91±06.79	91.03±03.22	89.11±05.16	74.60±02.95

B	Cytotoxic effects of PRE on cell lines (n=3, $\overline{X}$± S$\overline{x}$24 Hours)						
	6.25 µg/mL	12.5 µg/mL	25.00 µg/mL	50.00 µg/mL	100.00 µg/mL	200.00 µg/mL	400.00 µg/mL
RPE-1	92.68±04.49	84.61±06.41	77.45±06.74	71.91±06.22	63.65±07.54	54.53±05.23	48.69±08.40
MCF-7	88.85±10.16	84.12±12.46	77.67±12.25	70.13±09.31	70.57±08.82	62.08±06.57	54.29±04.65
U2OS	88.92±05.50	88.09±02.56	84.17±05.30	80.42±02.65	81.45±09.44	79.14±02.88	56.16±09.94

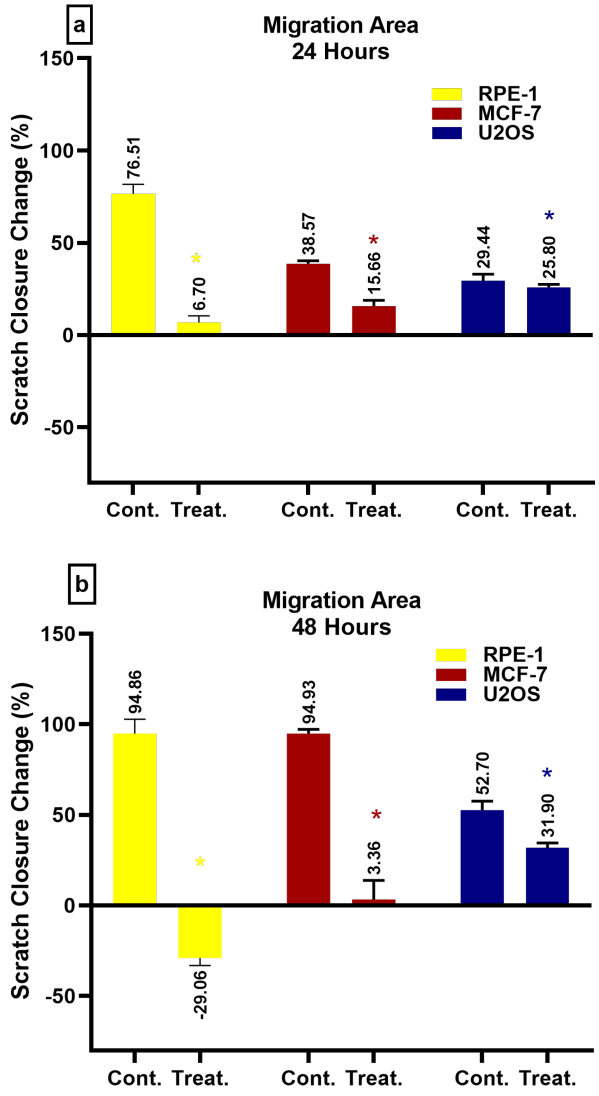

Figure 4. Migration rate of PRE on healthy and cancer cell lines at a: 24 and b: 48 hours (*p < 0.01 compared to their control groups).

When the results of the U2OS cell line were examined, the difference between the 24th hour control (29.44%) and treatment groups (25.80%) was found to be insignificant. However, when the data obtained at the end of the 48th hour were examined, the scratch closure rate in the control group and treatment group was found to be 52.70% and 31.90%, respectively, and this difference between the two groups was deemed statistically significant.

For RPE-1 cells, the ratios of the control and treatment groups at the end of 24 hours were 76.51% and 6.70%. At the end of 48 hours, the scratch was 94.86% in the control group and -29.06% in the treatment group.

Discussion

Testing novel biological, chemical, or plant extracts as stand-ins for currently available cancer therapeutic drugs has gained popularity in recent years. Studies have shown that PRE, one of these natural compounds, has anti-carcinogenic, anti-inflammatory, antiulcer, antidiabetic, and antioxidant effects. Tripathy et al. (2015) reported in their study that the survival of MCF-7 cells applied to PRE was significantly reduced [18]. Another study observed PRE's dose dependent growth inhibitory effect on the MCF-7 cancer cell line [34].

The results of our study, consistent with previous studies, revealed that PRE reduced the percentage of live MCF-7 breast cancer cells in a concentration-dependent manner. However, the cytotoxic effect of PRE obtained by the methanolic extraction method on the MCF-7 cell line was lower than the study conducted by Tripathy et al. This may be due to the extraction method.

In our investigation, dose-dependent PRE also decreased the viability of U2OS cancer cells. Nevertheless, MCF-7 cells were more sensitive than U2OS cells. This suppression of cell line growth can be attributable to either inhibition of cell proliferation or cell death, as a decrease in cell growth was observed with increasing PRE concentration in U2OS cells. In this investigation, we also found that normal cells were killed by PRE treatment at a rate comparable to that of cancer cells. This finding contradict previous research suggesting that PRE is less harmful to lymphocytes than cancer cells [18]. One reason for this

discrepancy is the possibility that the toxicity level of PRE varies in different types of normal cells [20].

Cancer cell mobility and metastasis are multifactorial processes involving different mechanisms [18, 35]. Because of this, metastasis remains one of the biggest challenges in successful cancer treatment. Preventing metastasis in cancer is an important goal for improving the patient's prognosis. No current information addresses the effects of PRE on cancer cell migration. However, the results of the wound healing assay in this investigation demonstrated that PRE slowed the migration of live MCF-7 and U2OS cancer cells and that these effects grew with time.

However, research conducted in vivo has shown that PRE is rich in polyphenolics and displays notable wound-healing action. This is due to their capacity to act as potent antioxidants and to encourage cellular re-epithelialization, cell proliferation, and collagen deposition [36, 37].

Contrary to what was found in wounds created in cutaneous tissues, PRE demonstrated anti-migration characteristics in the healthy cell line (RPE) in our investigation. This could be because different pathways were activated in different cells.

Conclusion

As a result, this study assessed PRE's cytotoxicity in MCF-7 and U2OS cells and discovered that it had a dose-dependent antiproliferative effect on these cells. Additionally, our research showed that PRE may limit the migration of two different cell types in vitro, indicating that PRE may be very useful in treating human osteosarcoma and breast cancer. More investigation is required to understand PRE's molecular mechanisms in cancer therapy fully.

Ethical approval

Since it is a cell study, ethics committee permission is not required.

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