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Original Article

The Toxicological effect of Cuscuta epithymum and Artemisia absinthium species on CP70 ovarian cancer cells

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ABSTRACT

Background: Ovarian cancer is one of the most common malignancies among women worldwide and is also the fifth leading cause of cancer death among women in recent years. Herbs have been used for centuries to treat a variety of ailments, including cancers. Medicinal plants can be use d in pure form or in combination with others.
Methods: In this study, after preparing the plant dry, extracts of *Artemisia absinthium* and *Cuscuta epithymum* were extracted by Soxhlet method. After Preparation of cell culture medium, Investigations continued. MTT test and Gap closure test were performed to evaluate the toxicity of *Cuscuta Epithymum* and *Artemisia Absinthium* Species on CP70 Ovarian Cancer Cells to Compared these two extracts mortality rate.
Results: The lowest cell migration in 48 h was related to *A. absinthium* treatments with concentrations of 1: 100 and 1: 1000. The lowest cell migration in the period of 72h is related to the treatment of *C. epithymum* with a concentration of 1: 1000, which these results are less significant than the effect of *A. absinthium*. The lowest cell migration in the period of 72h is related to the treatment of *C. epithymum* with a concentration of 1: 1000, which these results are less significant than the effect of *A. absinthium.*
Conclusions: MTT test showed that the treatment of plant extracts of *C. epithymum* and *A. absinthium* can disrupt the growth process as well as the migration and progression of CP70 cancer cells of ovarian cancer and cause cell apoptosis.
Keywords: MTT, drug plant, HIF1α, FGFRL1, GAPDH.

1. Introduction

Artemisia absinthium L. (Asteraceae) is a perennial herbaceous plant, 80-120 cm tall. It is a medicinal plant and has been used in traditional medicine to expel intestinal *A. absinthium* [1]. *A. absinthium* contains a compound called Artemisinin, which is the reason for the inability of cancer cells to survive in the presence of this plant [2]. Artemisinin has a high lethality and with the help of chemical proteomics, researchers have now shown

that artemisinin kills the malaria parasite by indiscriminately binding to proteins in many of the organism's key biochemical pathways [3]. Interestingly, it has nothing to do with healthy cells. This Asian medicinal plant has health-promoting properties. This plant can kill cancer cells in just 16 hours [4, 5].

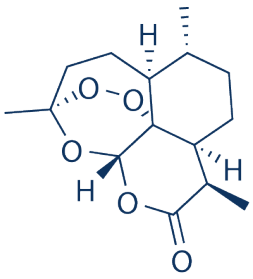

Figure 1. Chemical structure of Artemisinin [1].

Cuscuta epithymum (L.) (Convolvulaceae) is a parasitic plant that has long been used locally and traditionally in Eurasia. *C. epithymum* is an annual, twisting herbaceous plant that lives parasitically on a number of herbaceous plants [4]. In Iran, it is seen around Tehran, Karaj, Yazd, Isfahan, Fars, Hormuz Island, and Qeshm [6]. All parts of the plant have medicinal uses and its medicinal properties will depend on the host plant [7-9].

Cancer is currently the second leading cause of death in the Iran after cardiovascular disease, and its prevalence continues to rise [10, 11]. Ovarian cancer is one of the most common cancers in women and the fifth leading cause of death in women [12]. Although this cancer is common, for some reason it causes a lot of deaths [13]. One of the reasons for this is the vagueness of the symptoms of ovarian cancer, which doctors usually consider to be related to other common diseases. Another reason is that there is no effective diagnostic test for this disease [14]. Major regulator for cell differentiation, cell proliferation, and tissue polarity in embryonic development and homeostasis in adult tissue is the hedgehog (Hh) pathway [15].

Numerous studies have been done by laboratories reveal activation of this pathway in a variety of human cancer, especially ovarian cancers [16, 17]. Our goal in this research project is to investigate the genes involved in this pathway such as HIF1α, FGFRL1, and GAPDH [18]. Due to the importance of cancer diseases in this study, the role and effect of *C. epithymum* and *A. absinthium* plant species on the progression, cell migration, and expression of genes involved in the Hedgehog pathway in CP70 ovarian cancer cells have also been investigated.

2. Methods and materials

2.1. Materials and equipment

Ethanol was supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from Kimia Tehran acid co (Tehran, Iran). All materials related to Real-time PCR were provided by Pars Company. For this purpose, first HIF1α and FGFRL1 gene sequences were taken from NCBI site and according to the desired gene sequence, the primers were designed by PRIMER3 software and ordered to Pishgam Sanat Company for fabrication. Both PCR and Electrophoresis were purchased from Analytik Jena Biometra (Überlingen, Germany).

2.2. Plant preparation

100 grams of *Artemisia absinthium* cultivated in Isfahan province of Iran and 100 grams of *Cuscuta epithymum* dried and cultivated in Ilam province of Iran were prepared.

Figure 2. Images of *Cuscuta epithymum* on the left and *Artemisia absinthium* on the right

2.3. Extraction by Soxhlet method

50 grams of each plant completely into powder and used as samples. The ratio of solvent 70 (96% ethanol) to 30 (Ultrapure water) was considered. 100 mL of solvent were required for 10 grams of each plant. Four concentrations (pure, 1:10, 1: 100 and 1: 1000) were prepared using distilled water. Dilution operations were performed in the same way for both plants and kept in the laboratory refrigerator at +4 °C**.** The extracts were mixed with distilled water. Four concentrations including pure extract, 1:10, 1: 100 and 1: 1000 were also used for each extract[19, 20].

2.4. Preparation of cell culture medium

45 mL of RPMI medium was poured and 125 µl of penstrept (penicillin-streptomycin solution) was poured into RPMI medium and mixed. In the third stage, 12.5 microliters of Fanjizon were added to Penstrap. In the fourth step, 0.3 g of glutamine powder was weighed and added to the culture and it was well vortexed, the glutamine turned the medium yellow. 5 mL FBS was poured into a 50 mL Falcon with the help of a pipette. It should be noted that FBS does not need to be filtered (to create an environment with 10% FBS). The contents of RPMI, L-glutamine, Fangizone, and Penstrap were slowly filtered into Falcon containing FBS. After cell culture and cell passage, the cell counts were performed. Trypan blue solution 0.4% was used to dilute the cells during counting. Trypan blue is a vital dye, so the membranes of living cells do not allow it to pass through and repel it if they enter, but the membranes of dead cells are not able to remove the vital dye from the cytoplasm, and therefore the dead cells turn blue and easily recognizable from living cells that are not stained [18].

2.5. MTT test

The MTT test is a test to check the viability of a cell or evaluate the toxicity of drugs or other supplements on the cell, which can differentiate between living and dead cells by affecting intracellular organs. For this purpose, three plates of 96 sterile houses were prepared. The contents of the flasks were transferred to a 15 mL Falcon. The bottom cells of the flask were removed with the help of EDTA trypsin and poured into the falcon and onto the previous cells, and then centrifuged at 1000 rpm for 5 minutes. The amount of culture medium was calculated in relation to the number of treatments. Three plates of 96 houses were provided, each with 30 wells for MTT. Each well was filled with 100 µl of cell suspension. 100 µl of cell suspension was added to each well, containing 104 × 1 cells. Seventy microliters of extracts were poured in a certain concentration into each well. After adding the

treatments to all three plates, they were incubated in the incubator for a specified time. After 24 hours, a plate for 24 hours treatment was removed [21].

Each 24-hour plate well was filled with 10 µl of MTT solution and gently pipetted to mix the solution with the entire contents of the wells and then incubated for 3.5 hours in an incubator at 37 ° C. After the desired time, 50 microliters of Detergent Reagent solution was added to it. After about half an hour of incubation at room temperature, light absorption was read at 570 nm and 630 nm with the help of ELISA. The same procedure was repeated in the following days for 48-hour and 72-hour plates, and the absorptions were taken with ELISA, and the results were averaged for each of the concentrations of the extracts, and notes are given in the following tables. It should be noted that *Artemisia absinthium* with symbol A and *Cuscuta epithymum* with symbol B have been reported. The adsorption of the treatments was assessed by ELISA at 570 and 630 wavelengths. In the next step, the percentage of cell death and survival for each concentration was calculated separately according to the following equations:

Survival rate = (sample absorption at 570 nm - sample absorption at 630 nm) * 100

2.6. Gap closure test

One of the most economical and efficient methods of studying cell migration in vitro. This method is based on creating an artificial gap on the surface of a cell surface layer with high surface density, the cells of the edge of the scratch area move towards each other until they finally close this gap through cell-to-cell connections. One of the benefits of this method is the simulation of normal cell migration in vitro [22].

3. Results

3.1. Extraction

The total weight of the dry extract obtained from *Artemisia absinthium* is 7.29 g of 50 g of the dry extract of this plant, ie about 14.5% of the weight of the original plant extract, and the total weight of the dry extract obtained from *Cuscuta epithymum* is 6.74 per 50 g of dried fruit, ie about 13.48% of the extract was obtained**.**

3.2. Cell count

According to the results and calculations obtained from the neobar slide, the number of cells counted in a 15 mL flask equal to 107 1.8 cells cells / mL was obtained.

3.3. Cell Scratch Assay Analysis

3.3.1. Migration of CP70 cells treated with *Artemisia absinthium* extract in 24, 48 and 72 hours

Cell migration analysis was performed in groups controlled and treated with *A. absinthium* by Cell Scratch Assay. The lowest cell migration in the 24h period was related to the *A. absinthium* treatment with a concentration of 1: 1000 ($p < 0.01$). The lowest cell migration in 48 h was related to *A. absinthium* treatments with concentrations of 1: 100 and 1: 1000 ($p < 0.01$). The lowest cell migration in the period of 72h is related to *A. absinthium* treatments with concentrations of 1: 100 and 1: 1000 ($p < 0.01$).

These results confirm the results of MTT, the thinnest concentration of *A. absinthium* extract, which has the highest uptake into cancer cells and has the highest cell death. It should be noted that all concentrations of *A. absinthium* extract prevented the migration of CP70 cells to the scraper.

3.3.2. Migration of CP70 cells treated with *Cuscuta epithymum* plant extract in 24, 48 and 72 hours

Cell migration analysis was performed in control and *Cuscuta epithymum* - treated groups by Cell Scratch Assay at 24-hour treatment. The lowest cell migration in a 24-hour period is related to *C. epithymum* treatment with a concentration of 1: 1000. The lowest cell migration in the period of 48h is related to *C. epithymum* treatment with a concentration of 1: 1000, which has less significance than the effect of *Artemisia absinthium* ($p < 0.01$). The lowest cell migration in the period of 72h is related to the treatment of *C. epithymum* with a concentration of 1: 1000, which these results are less significant than the effect of *A. absinthium* ($p < 0.01$).

These results confirm the results of MTT, the thinnest concentration of *C. epithymum* extract, which has the highest uptake into cancer cells and has the highest cell death. It should be noted that all concentrations of *C. epithymum* extract prevented the migration of CP70 cells to the scraper.

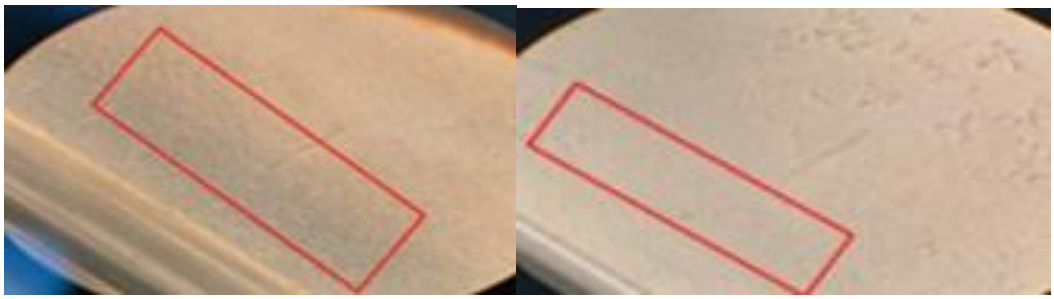

Figure 3. Evaluation of CP70 cell migration treated with *Artemisia absinthium* (right) and *Cuscuta epithymum* (left) after 72 hours.

3.4. Crystal violet staining analysis

Progressive results in CP70 cells treated with different concentrations of *Artemisia absinthium* and *Cuscuta epithymum* extracts at intervals of 24 to 72 hours by isolation, fixation and staining of these cells by violet crystal dye and adsorption of stained cells by ELISA Reader in The wavelength of 570 nm indicates the concentration of cells with progressive activity in the scratch area. According to Tables, the rate of CP70 cell uptake, depending on the uptake obtained, indicates that almost all concentrations of *A. absinthium* extract in the treatment hours of 24, 48 and 72 hours inhibited the progression of cells. But 1: 1000 *A. absinthium* extract showed the least progress in 48 and 72h treatment hours ($p < 0.01$). Similar results were observed at a concentration of 1: 1000 *C. epithymum* extract at 24 and 48 hours ($p < 0.05$). Therefore, according to the results of the ELISA, the highest progression is for the net concentration of *C. epithymum* plant and the lowest progression is for the concentration of 1: 1000 *A. absinthium* after 72 hours of migration.

Table 1. Staining analysis of CP70 cells to obtain progressive uptake in the treatment groups

	PURE A	1:10 A	1: 100 A	1:1000 A	PURE B	1:10 B	1: 100 B	1:1000 B
24 h	0.104	0.147	0.117	0.107	0.195	0.119	0.141	**0.099**
48 h	0.107	0.120	0.111	0.102	.209	0.127	0.103	**0.108**
72 h	0.081	0.116	0.127	0.099	0.176	0.133	0.118	**0.132**

4. Discussion

Shafi in 2012, study the anti-proliferative property of *Artemisia absinthium* and *Cepcuta epithymum* were reported [23]. Noureen in 2019, study the effect of methanolic extract of *C. epithymum* was investigated and its antibacterial and anti-cancer effects were confirmed and further studies were suggested. Some species of *Cuscuta* L., including *C. reflexa*, are known to be anti-cancer and are used to treat prostate cancer, although there is still insufficient evidence[24].

Akbar in 2020, study the effect of chloroform and hydroalcoholic extracts of *C. epithymum* and *C. chinensis* on different cell lines (MB - MDA-468, HT29 and Hela) was investigated and the results showed that the extracts of the shoots of these two types of *C. epithymum* have properties [25, 26]. They are cytotoxic and are the best candidates for further studies to obtain new cytotoxic agents. Numerous studies have shown that the pharmacological effects of different species of *C. epithymum* are attributed to their active compounds, including the phallonoid polysaccharides and lignans [27].

Phalonoids are a variety of antioxidants, and polysaccharides are effective compounds in modulating the immune system. Studies have shown that the use of special types of polyhydroxyphenols, such as flavonoids, reduces the risk of colon and breast cancer. The human diet contains a mixture of plant polyphenols. Various studies indicate that these phenols have cytotoxic effects against various tumors and the mechanism of action of these compounds is through induction of apoptosis [28]. A study was conducted on the effect of herbs on inhibiting bacteria and the p53 gene, which showed that those who were exposed to and used herbs had a longer lifespan than those who were not**.** So barberry is a potential anti-cancer supplement [29]. A study by Betancur-Galvis in 1999, examined the antitumor and antiviral activity of Colombian plant extracts such as pineapple and its root, and concluded that compounds such as Acetogenins are potent inhibitors and the methanolic extract of *Annona* sp. had a more acceptable activity than extracts of other species [30].

Rhode in 2007, found that ginger extract reduced the growth of ovarian cancer cells and moderated angiogenic factors [31]. Mansour in 2019, also showed ginger fat in anti-cancer mice, and a study showed the killing of gingerols on ovarian cancer cells [32]. In various studies, ginger extract has been shown to inhibit skin cancer in mice and rats. This substance causes apoptosis in ovarian cancer cells in mice. Fresh aqueous ginger extract has also been shown to have a cytotoxic effect on breast cancer cells [18, 20].

5. Conclusion

Finally, based on the results of this study and other studies, it can be concluded that the *Artemisia absinthium* extract affect reducing migration, progression due to MTT growth assays in ovarian cancer.

Abbreviation

FBS: Fetal bovine serum
MTT: Mean Transit Time
PCR: Polymerase chain reaction

Conflict of interest

We have no conflicts of interest to declare

Consent for publications

Ameneh Javid, Saeed Rezaei Zarchi, and Homa Hakimian read and approved the final manuscript for publication.

Availability of data and material

We Embedded All Data In The Manuscript**.**

Authors' contributions

The experiments and writing were done by Ms. Hakimian and the experiments were designed by Dr. Rezaei Zarchi and finally the study was supervised by Dr. Javid.

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Ethics approval and consent to participate:

In this study, no animal or human samples were used and the whole process was performed under the protocol of the University Ethics Committee

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