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Assessing the Anticancer Effect of the *Euphorbia condylocarpa* Plant on AGS Gastric Cancer Cell Line

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Abstract

Background: Spurge family is a large family of plants that contains more than 300 genera and 800 species of which 5 genera and 72 species are native to Iran. This important family of plants can be used in various disease treatments such as cancer, arthritis, asthma, bacterial infections, and neuralgia. This study investigated the anticancer effects of the alcoholic extract of *Euphorbia condylocarpa* whose native Iranian Kurdish name is "Shoalehkolah".
Methods: In this study, the cytotoxicity of the methyl extracts of *Euphorbia* was performed on AGS gastric cancer cell lines using the MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) assay. The cytotoxicity of the methyl extract of *Euphorbia condylocarpa* with dilutions of 10, 20, 100, and 200 μg/mL was considered.
Results: The obtained data showed that the greatest effect was related to the dilution of 200 μg/mL.
Conclusions: The findings suggest that the methyl extract of this plant can be considered for its anticancer effects.

Keywords: Gastric cancer, Anticancer, *Euphorbia condylocarpa*

1. Background

Gastric cancer is one of the most common malignancies worldwide (1). In Iran, the mortality rate of gastric cancer is the first cause of death due to cancer in both sexes (2). According to the statistics, it is important to find new ways of treatment. So, one of the major challenges in medical prevention is an effective and timely treatment of cancer. Due to the non-specific effects of anti-cancer drugs and their damage to other healthy tissues of the body, natural inexpensive gradients with fewer side effects are needed. Screening the studies on different species of *Euphorbia* shows beneficial therapeutic effects on various diseases such as cancer, arthritis, asthma, bacterial infections, and nerve pain (3). Some researches into the effects of various compounds have demonstrated the effects of various compounds like diterpene (4), triterpene (5), ricin (6), and esters (7). Several studies have reported the anti-bacterial effects of some species of *Euphorbia* like fosiformis (8), *E. heterophylla* (9), and *hitra* (10). Also, there are different reports about the antifungal activities of some species of *Euphorbial* like *E. tirucalli* and *E. hitra* (11). *Euphorbia condylocarpa* (local name is Shoaleh Kolah) that exists in Iran (12, 13) is one of the major species of *Euphorbia*. Further distribution of this plant is in Kurdistan province and, also, it is really common as an effective drug to treat constipation and intestinal infection (14).

Brief studies have been conducted about *Euphorbia* and its possible effects on disease, but none of them has been about its anticancer effects in Iran and other parts of the world.

2. Objectives

The purpose of this research is a more comprehensive study about the anticancer effects of this native Iranian plant and identifying and extracting the substance causing this feature to achieve a natural anticancer drug.

3. Methods

3.1. Collection of Plants and Extraction

The root of *Euphorbia* was collected from surrounding mountains in Kurdistan in the west of Iran and the root parts of the plant were dried at room temperature in the shadow for 14 days. Extraction was performed using the percolator and percolation method. 250 grams of the

dried root was grinded and placed into the percolator machine and, then, it was covered with 500 ml of 5.99 percent methyl alcohol. After acquiring a few drops of solvent, percolation procedure was performed for 72 hours. Extraction procedure was continued until the existence of whole colored solvent.

3.2. Evaluating the Anticancer Effect of the Extract

Approximately 5×10^4 cells were cultured per well and were confirmed by cell counting using Trypan blue. After culturing the cells, 96 well plates were incubated for 24 hours at 37°C until 70 percent of the wells got filled. For the manipulation of cells, different dilutions were prepared in sterile micro tubes and different values (0, 10, 100, and 200 μg/mL) of the extract were prepared by 1% DMSO solvent. Then, 100 μL of each dilution was added to the wells and three wells were considered for each dilution. Medium and drug were changed after 24 hours and 100 μL of RPMI medium were added and, after 24 hours, the plates were observed by the microscope. Then, 25 μL of MTT solution was added into the plates in darkness and they were placed in an incubator for about 2 to 4 hours. The supernatant was removed from the wells and 125 μL of 1% DMSO solution was added to the wells. Then they were vibrated for 20 minutes (away from the light) and the color intensity was measured at 570 nm wavelength.

3.3. Statistical Analysis

Results were analyzed using SPSS 15. Student's t-test has been used for the comparisons between the two groups. P value of less than 0.05 has been considered statistically significant.

4. Results

The cytotoxicity evaluation of *Euphorbia* plant extract in different dilutions (zero as a control, 10, 20, 100 and 200) on AGS gastric cancer cell lines showed the most cytotoxicity effect on the concentration of 200 μg/mL and the minimal effect on the concentration of 10 μg/mL. Actually, the cytotoxicity increases by using the methyl extract of *Euphorbia condylocarpa* (Figures 1, 2 and Table 1).

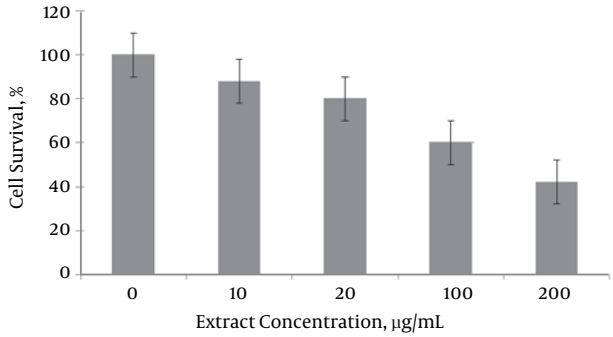

Figure 1. Column Chart Related to the Effect of *Euphorbia condylocarpa* Methyl Extract on AGS Cell Lines by MTT.

5. Discussion

These days, due to poor lifestyle, cancer has spread widely throughout the world. According to studies, 3 to 4 children out of each hundred thousand children worldwide get affected by cancer annually (15) and, also, due to the rapid growth of cancer cells, anticancer drugs are designed to collate with them. Considering that some normal cells including digestive system, reproduction, follicular, and blood cells multiply at a rapid rate, normally they get affected by these drugs and this is the reason for their side effect.

On the other hand, these drugs are so expensive and using them is so costly for patients. Studies on *Euphorbia condylocarpa* and its possible effects on the disease have been very insignificant and no investigation has been conducted on the anticancer effects of *Euphorbia condylocarpa* plant. Therefore, this investigation was tried to be a more comprehensive study of the anticancer effects of this native Iranian plant. Because of the importance of medicinal plants to obtain medication, studying the efficacy, safety, and the features of medicinal plants is very important (16). Some medicinal plants contain biologically activated components and have antimicrobial and anticancer properties. They have been used for the treatment of a variety of diseases over the years (8). These medicinal plants, due to their low cost, easy access, and experiences which are inherited from the ancestors constitute valuable components of the traditional medicine (17) and a family of plants that have been studied more about is *Euphorbia*. Roshchin et al. (18) found a substance wih the nature of B-euphorbal and triterpene in *E. condylocarpa* and also B-Euphorbal was previously found in *Euphorbia* (19).

In the following physical and chemical studies on methyl extract, a substance called naringenin 7-O-B-D glucopyranose was isolated for the first time and it was the first report for *Euphorbia* genus plants (18). The assessment of the impact of different dilutions of *Euphorbia condylocarpa* methyl extract on AGS gastric cancer cell lines showed the most cytotoxic effect at the concentration of 200 μg/mL and the minimal effect on the concentration of 10 μg/mL and, also, by increasing the methyl extract, cellu-

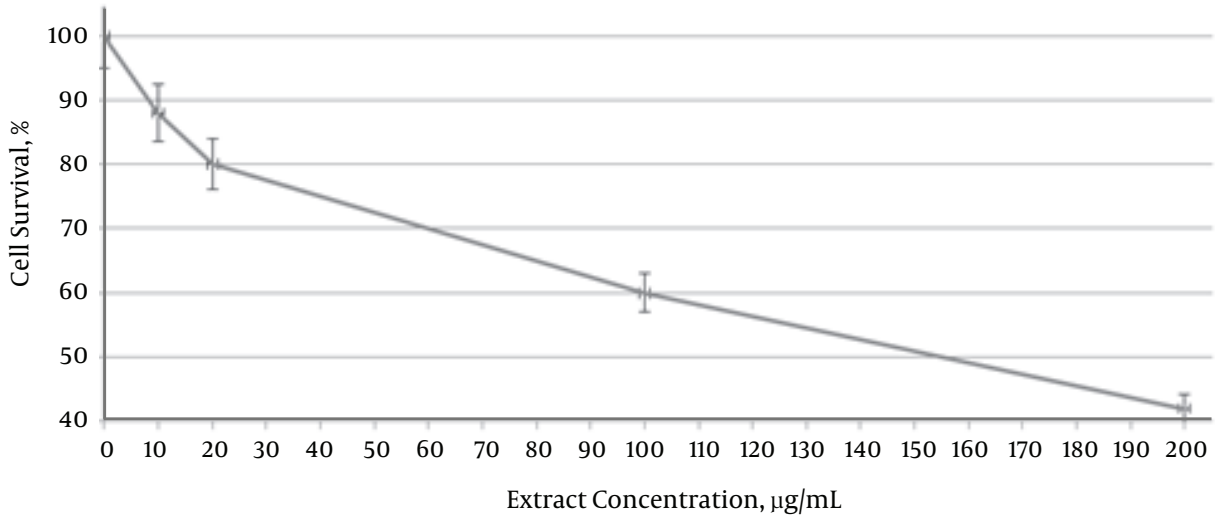

Figure 2. The Effectiveness Trend of *Euphorbia condylocarpa* Methyl Extract on AGS Cell Lines by MTT

Table 1. Cytotoxicity Evaluation of *Euphorbia condylocarpa* Methyl Extract on AGS Cell Lines by MTT

	1	2	3	4	5
Dose (μg/mL)	0	10	20	100	200
Viability %	100	88	88	60	42

lar cytotoxicity increases. But, in another study, the results of MTT test on *Euphorbia helioscopia* which was observed in SMM C-7721 early apoptotic cells, the maximum effect was due to 200 μg/mL (20). In this study, the most anticancer effect has been in 200 μg/mL, so the same effective trend can be seen. The results in comparison with the results of Amirghofran et al. in 2006 showed the most inhibitory effect of jurkat cells in 5.12 mg/ml dilution (21).

According to the survey conducted in this study, it can be stated that *Euphorbia condylocarpa* methyl extract has an anticancer effect on gastric AGS cell lines; although, more studies are needed. MTT assay is only a preliminary test done for screening and does not confirm the anticancer activity completely. Further researches should be done with evaluation techniques like flow cytometry and invitro bioassays to confirm the anticancer effects of *Euphorbia condylocarpa*.

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