The Hydroalcoholic Extract of Pistachio Kernel and Pericarp has an Inhibitory Effect Against Prostate Cancer Cells

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Abstract

Introduction: Pistachio contains compounds with antioxidant activity. Due to the high phenolic and antioxidant content of different parts of pistachio, including leaves, gum, pericarp, and seed, researchers have concentrated on this plant to research its anti-cancer activity. This paper aims to investigate the anti-proliferation effect of pericarps and kernels of *P. vera* var. *akbari* on the PC-3 prostate cancer cells.

Materials and Methods: In this study, the effect of hydroalcoholic extract of pistachio pericarps and kernels on the proliferation of prostate cancer cells was measured by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For this purpose, culture media of prostate cancer cells were treated with final doses (0, 0.1, 0.5, 1, 2.5, 5, 10 mg/mL) of extract for 24, 48, and 72 h, and the IC50 values were measured. All experiments were performed in triplicate with three replications.

Results: This research results indicated that both extracts reduced cell viability in cancerous cells in a dose- and time-dependent manner significantly. The IC50 values of the hydroalcoholic extract of pistachio pericarp against PC-3 prostate cancer cells after 48 h and 72h treatment were 8.73 and 4.24 mM, respectively. However, these values for pistachio kernel extract were 6.52, 4.72, and 4.41 mM, respectively. Data were analyzed by a one-way ANOVA, and a value of $P<0.05$ was considered statistically significant.

Conclusion: The results demonstrate the anti-cancer activity of pistachio seed and pericarp on human prostate cancer cells in *in vitro*. The anti-carcinogenesis effects of pistachio are probably related to the presence of a considerable number of biologically active components, including phenolic compounds with antioxidant properties.

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1. Introduction

Cancer can be described as a multifactorial inheritance disorder and a global public health issue; it is also considered the second major cause of death mortality worldwide, imposing a high cost to health systems [1]. Using novel treatments, including chemotherapy with new drugs, surgery, and radiation therapy, has improved the survival rate of cancer patients. However, secondary illnesses and metabolic imbalances, such as malnutrition, systemic oxidative stress, and inflammatory state, have been usually caused by these interventions. This can justify why patients increasingly seek alternative and complementary medicine therapies to cope with side effects resulting from cancer treatment [2].

Oxidative stress normally results from reactive oxygen species (ROS)[3,4]. Peculiarly, they are responsible for the proliferation, intracellular signaling, and survival in cancerous and normal cells. Oxidative stress in the cell caused by reactive oxygen species leads to the instability of nucleic acids, lipids, proteins, and other biomolecules [5]. This essential balance can justify the genomic integrity, integrative metabolism, immunity, and internal-external signaling of normal and cancer cells [5]. Cells have a combined antioxidant system to deal with internal oxidative stressors, binding an external protective core (e.g., vitamin C) to antioxidant compounds and molecules such as glutathione [6]. Various types and amounts of phytochemicals, which display distinct antioxidant potential, are found in fruits and vegetables. The antioxidant properties of more than 3,000 high-consumption foods in the human diet have been reported. Research shows that plant-based foods have 8 to 9 times the higher antioxidant capacity than animal foods [7]. The amounts and types of antioxidants determine the way plant foods can be classified. For example, ascorbic acid, carotenoids, and tocols are abundantly found in citrus fruits, vegetables, and seeds, respectively. Tocols, including tocopherols and tocotrienols, can be found in some plant foods, such as nuts and oilseeds [8]. They have anti-cancer effects in several cancerous cell lines, including PC3 and LNCap [9].

Pistachio (*Pistacia vera*) is known for its high content of polyphenols. It contains powerful and well-known antioxidants with potentially protective effects against diseases caused by the overproduction of free radicals, such as CVD and cancer [10].

Due to the substantial phenolic and antioxidant content of different parts of pistachio, including leaves, gum, pericarp, and seed, researchers have concentrated on this plant to research its anti-cancer activity. The current paper aims to

investigate the anti-proliferation effect of pericarps and kernel of *P. vera* var. *akbari* on the PC-3 prostate cancer cells.

2. Materials and Methods

2.1. Materials

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit, Penicillin-Streptomycin, Trypsin-EDTA solution, RPMI 1640 culture medium, and Fetal Bovine Serum (FBS) were obtained from Shellmax (China). The PC3 prostate cancer cell line was prepared from the Pasteur Institute, a national cell bank of Iran.

2.2. Preparation of hydroalcoholic extract of pistachio pericarps and kernels

During the pistachio harvest in Rafsanjan city of Iran, fresh *P. vera var. akbari*, not been sprayed for a long time, was purchased from gardeners. Then, the fresh pericarps and kernels of pistachio were separated and dried in the shade. They were then powdered using a grinder. To prepare the hydroalcoholic extract from the pistachio pericarps and kernels, 150 g of them were weighed separately and soaked in 70% ethanol, being continuously stirred at room temperature for 24 h; then, they were filtered. The filtered hydroalcoholic extract was evaporated in a freeze-dryer overnight. The dried extract was retrieved and kept at −20°C for later usage.

For the preparation of 100 mg/mL stock solution, the hydroalcoholic extract was solubilized in dimethyl sulfoxide (DMSO); it was then freshly diluted with the culture media to gain the working concentrations (0, 0.1, 0.5, 1, 2.5, 5, 10 mg/mL).

2.3. Cell culture

RPMI 1640 containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin were used to culture cell line PC-3 of human prostate cancer in a humid incubator with 5% CO2.

2.4. Treatment of prostate cancer cells with the hydroalcoholic extract of pistachio pericarps and kernels

6×10^4 cells were cultured to determine the effects of the hydroalcoholic pistachio pericarps and kernels extract on the cancer cells. Subsequently, final extract concentrations (0, 0.1, 0.5, 1, 2.5, 5, 10 mg/mL) were applied for 24, 48 and 72 h. All experiments were performed in triplicate with three replications.

2.5. Cell viability assay

The effects of hydroalcoholic extract of pistachio pericarps and kernels on the proliferation of prostate cancer cells were determined using the MTT assay. In short, the cells were plated with a concentration of 5×10^4 cells/well in 100 µL of total culture medium with final concentrations 0, 0.1, 0.5, 1, 2.5, 5, 10 mg/mL of freeze-dried hydroalcoholic extract of pistachio pericarps and kernels in 96-well ELISA plates. Next, 10 µL of MTT with 5 mg was

added to each well, and they were incubated for 2 h at 37°C. Then, the plate was centrifuged at 600g for 5 min to remove MTT from the wells. After removing the MTT solution, each well received 100 µL of DMSO, and plates were shaken for about 10 min at room temperature. Finally, the optical density (OD) was recorded on an ELISA reader at a wavelength of 570 nm. The following formula was used to calculate cell viability:

Cell viability = (OD test/OD control) × 100.

2.6. Statistical analysis

Statistical analysis was performed by SPSS Version 18.0. Further, differences between groups were determined using a one-way ANOVA and Student's t-test by comparing different concentrations of hydroalcoholic extract of pistachio pericarps or kernels with the control group. Results were reported as means±SD, and a value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Effect of hydroalcoholic pistachio pericarp extract on the cell viability of PC-3 prostate cancer cells

The growth suppression effects of the hydroalcoholic pistachio pericarp extract on PC-3 prostate cancer cells were examined using the MTT assay. The cancer cells were treated with various doses of hydroalcoholic pistachio pericarp extract (0, 0.1, 0.5, 1, 2.5, 5, 10 mg/mL) for 24 h (Fig. 1), 48 h (Fig. 2), and 72 h (Fig. 3). The findings revealed that the extract reduced cell viability in cancerous cells, in a dose- and time-dependent manner, significantly ($P<0.05$).

The IC50 values of the hydroalcoholic pistachio pericarp extract against PC-3 prostate cancer cells after 24 h, 48 h, and 72 h treatment were measured, as presented in Table 1.

The cytotoxicity effect of pistachio pericarp extract against PC-3 prostate cancer cells was observed with more than 60% and 70% inhibition after 48 h (Fig. 2) and 72 h (Fig. 3) treatment, respectively.

Table 1- Demonstrates the IC50 values of the hydroalcoholic extract of pistachio kernel and pericarp against PC-3 prostate cancer cells in 24 h, 48 h, and 72 h after treatment

The hydroalcoholic extract	IC50(mM)		
	24h	48h	72h
Pericarp		8.73	4.24
Kernel	6.52	4.72	4.41

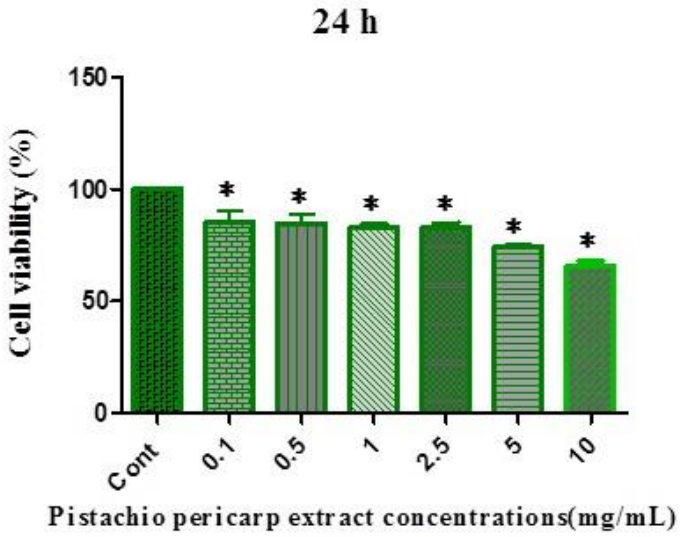

Figure 1- Demonstrates the viability changes in PC-3 prostate cancerous cells treated with various concentrations of the pistachio pericarp's hydroalcoholic extract after 24 h. The cell viability is determined using the MTT assay. Each column is an average of data from three different tests carried out in triplicate and presented as Mean ± SD

*= significant in compared with Con group (P< 0.05)

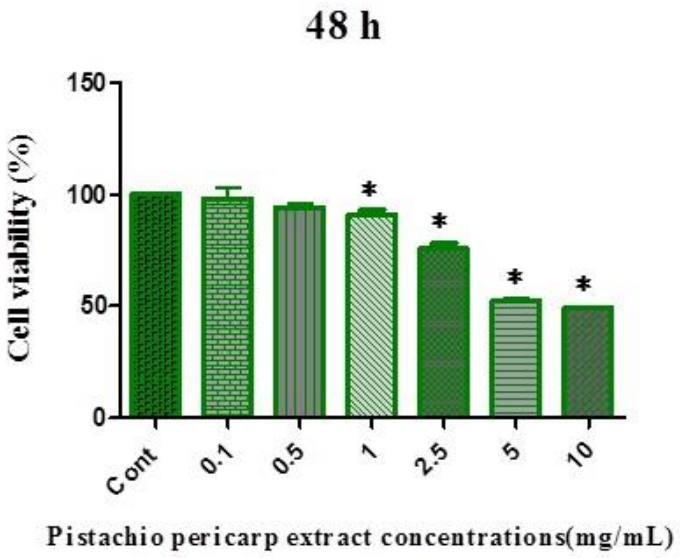

Figure 2- Illustrates the viability changes in PC-3 prostate cancerous cells treated with various doses of the pistachio pericarp's hydroalcoholic extract after 48 h. The cell viability is determined using the MTT assay. Each column is an average of data from three distinct test experiments carried out in triplicate and shown as Mean ± SD

*= significant in compared with Con group (P< 0.05)

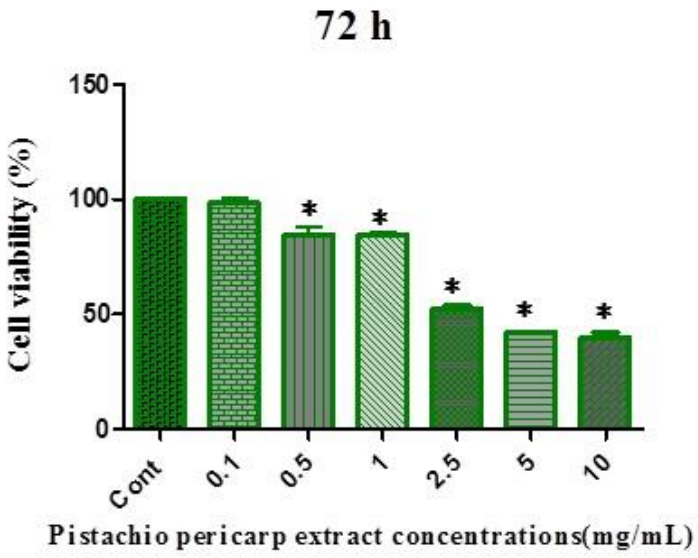

Figure 3- Depicts the viability changes in PC-3 prostate cancer cells treated with various doses of the pistachio pericarp's hydroalcoholic extract after 72 h. The cell viability is determined using the MTT assay. Each column is an average of data from three different tests done in triplicate and shown as Mean ± SD

*= significant in compared with Con group (P< 0.05)

3.2. Effect of hydroalcoholic pistachio kernel extract on the cell viability of PC-3 prostate cancer cells

The growth suppression effects of the hydroalcoholic pistachio kernel extract on PC-3 prostate cancer cells were examined using the MTT assay. These cells were treated with various doses of hydroalcoholic pistachio kernel extract (0, 0.1, 0.5, 1, 2.5, 5, 10 mg/mL) for 24 h (Fig. 4), 48 h (Fig. 5), and 72 h (Fig. 6). According to the results, the extract reduced cell viability in cancerous cells, in a dose- and time-dependent manner, significantly ($P<0.05$).

The IC50 values of the hydroalcoholic pistachio kernel extract against PC-3 prostate cancer cells, following 24 h, 48 h, and 72 h treatment, were measured, as provided in Table 1.

The cytotoxicity effect of pistachio kernel extract against PC-3 prostate cancer cells was observed with more than 60% and 65% inhibition after 48 h (Fig. 5) and 72 h (Fig. 6) treatment, respectively.

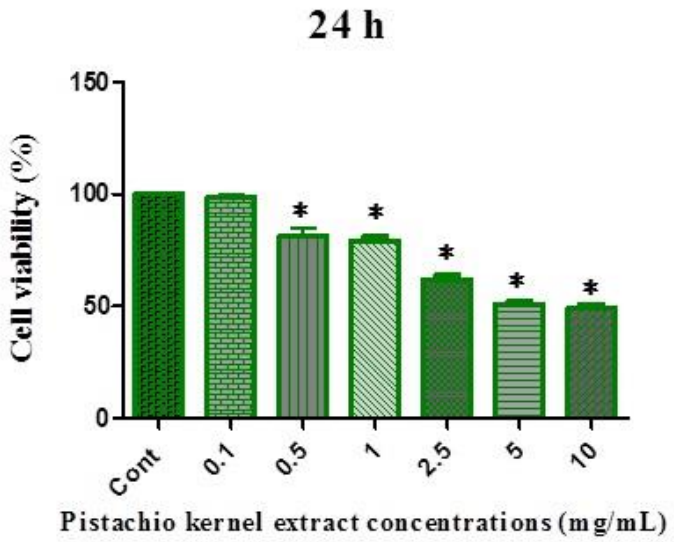

Figure 4- Illustrates the viability changes in PC-3 prostate cancer cells treated with various doses of the pistachio kernel's hydroalcoholic extract after 24 h. The cell viability is determined using the MTT assay. Each column is an average of data from three independent tests carried out in triplicate and shown as Mean ± SD

*= significant in compared with Con group ($P< 0.05$)

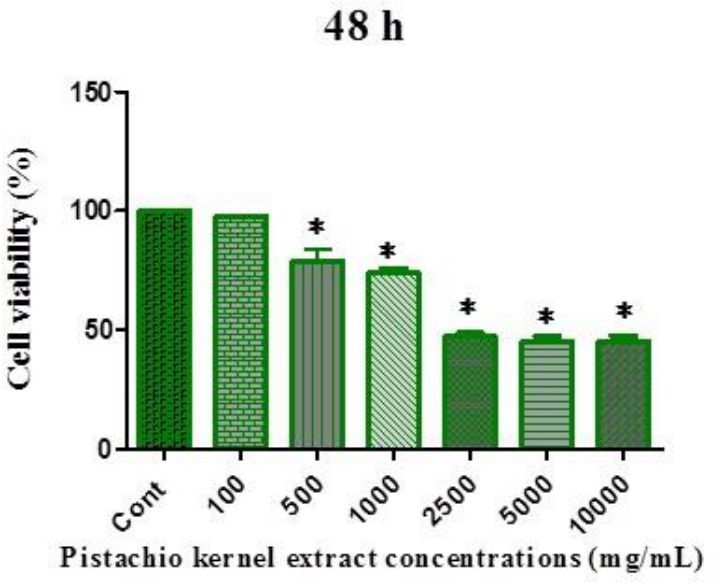

Figure 5- Shows the viability changes in PC-3 prostate carcinogenic cells treated with various doses of the pistachio kernel's hydroalcoholic extract after 48 h. The cell viability is determined using the MTT assay. Each column is an average of data from three different tests carried out in triplicate and presented as Mean ± SD

*= significant in compared with Con group (P< 0.05)

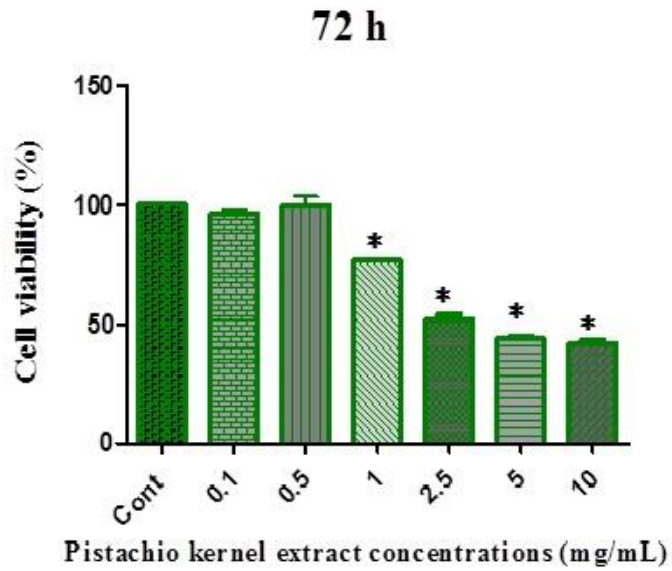

Figure 6- Illustrates the viability changes in PC-3 prostate cancerous cells treated with various doses of the pistachio kernel's hydroalcoholic extract after 72 h. The cell viability is determined using the MTT assay. Each column is an average of data from three independent tests carried out in triplicate and presented as Mean ± SD

*= significant in compared with Con group (P< 0.05)

4. Discussion

Pistachio (*Pistacia vera* L.) is considered one of the most important tree nuts worldwide. It is mainly planted in Mediterranean countries, saline arid parts of the Middle East, and America [11].

Pistachio contains valuable phenolic components, thus can be called a "unique functional food." Further, according to the latest ranking, it is among the first 50 foodstuffs with high antioxidant activity[12]. Pistachio nut contains several phenolic compounds, such as anthocyanins, proanthocyanidins, flavan-3-ols flavonols, stilbenes, flavanones, isoflavones, and phenolic acids, with high antioxidant activity [13], as well as cardioprotective, chemopreventive, and vasoprotective capacities [14]. For instance, anthocyanins, liable for the red color of some fruits and beverages (red wine, blueberries, orange), are shown to have anti-inflammatory, antioxidant, anticarcinogenic [15,16], and antiangiogenic potentials [17]. Isoflavones, in addition to their antioxidant attributes, have some chemopreventive activities due to a partial agonists role that they play at the level of estrogen receptors [18]. Therefore, isoflavones, such as genistein, appear to be basically accountable for the reduction in the prevalence of uterus and breast cancer in the female population of Asian countries, who mainly consume a soy-based diet rich in isoflavones [19].

Tomaino et al., 2010, have shown the antioxidant activity in pistachio skins partly resulted from the considerable amount of antioxidant components, including catechin, cyanidin-3-O-galactoside, gallic acid, eriodictyol-7-O-glucoside, epicatechin, and unidentified compounds. In addition, the results reveal that the great antioxidant activity of pistachio stems from the pistachio skin. Pistachio skin contains a higher amount of all phenolic groups compared to its seeds. It is noteworthy that anthocyanins (a substance responsible for the color in fruits and vegetables) are only found in pistachio skin, having excellent antioxidant activity [10].

Phenolic compounds are massively found in pistachio skin and pericarp. As secondary metabolites, they are found in some plants, with various nutritional properties, including antimicrobial and anti-cancer [20]. These properties probably result from their key role in decreasing oxidative stress in organisms [21]. The biological, medicinal, and therapeutic effects of pistachio pericarp and seed are associated with their radical scavenging and antioxidant activities [22-24].

The present research results indicate the anti-proliferation effect of pistachio seed and pericarp justified by its high antioxidant content. In this study, pistachio

kernels are used without being peeled in experiments. According to the literature, pistachio skin is liable for its considerable antioxidant activity. Therefore, due to its nutritious and beneficial properties, pistachio kernel is recommended to be consumed with skin.

5. Conclusion

This research contributes to the investigation of the anti-proliferative effect of pistachio seed and pericarp on prostate cancer cells. Indeed, the anti-cancer activity of pistachio seed and pericarp on prostate cancer cells in *in vitro is demonstrated*. The anti-carcinogenesis effects of pistachio are probably due to the presence of a great number of biologically active components, including phenolic with antioxidant compounds. Evidence shows that plant-derived polyphenolic antioxidants have a key role in controlling cancer, which is in line with the present study. According to the present research findings, people are recommended to use unpeeled pistachios in their meals. Further, pistachio skin as a by-product with anti-cancer properties in the food industry should be considered. Finally, pistachio pericarp, a discarded product, has the potential to be used in the pharmaceutical industry to produce anti-cancer drugs.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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