# Potential Anticancer Activity of the Genus Pistacia through Apoptosis Induction in Cancer Cells

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<table>
<thead>
<tr>
<th>Information</th>
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<td><strong>Article Type:</strong> Review Article</td>
<td>Cancer is one of the most significant global challenges threatening health. Accordingly, cancer management is one of the most important issues in the world. Evading apoptosis is a route through which a cancerous cell becomes malignant. Thus, designing novel apoptotic drugs against cancer is of high importance because deficiencies in the regulation of apoptotic pathways lead to cancer chemotherapy resistance. Apoptosis can be induced by inhibiting anti-apoptotic factors or stimulating pro-apoptotic molecules. On the other hand, chemotherapy complications have caused medical plants to be considered as potential alternatives for the treatment of tumors. Pistachios have been proved to have a wide range of pharmacological benefits, including anti-microbial, anti-oxidant, and anticancer properties. Evidence shows that anticancer effects of pistachios result from their influence on numerous apoptosis-related pathways in tumor cells. In this paper, we aim to introduce anticancer properties of pistachios, particularly those connected with targeting apoptosis-related pathways.</td>
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1. Introduction

Cancer is one of the most serious health challenges in the world. In the human body, new cells are frequently produced to repair damaged tissues. Under normal circumstances, the death and proliferation process of cells takes place in a balanced way. However, in tumor cells, the growth, division, and death of cells are irregular. Tumor formation occurs as a result of this irregularity [1, 2]. Apoptosis, i.e. programmed cell death, is controlled by various proteins and genes categorized into apoptotic proteins and genes. The former has a positive effect on apoptosis, which makes the cellular process further progress, yet the latter has a negative effect that blocks apoptosis [3]. Although apoptosis induced by chemotherapy is the main mechanism of various anticancer therapies, many of the drugs used have had different side effects and treatment resistance [4]. Understanding mechanisms associated with apoptosis is important for discovering novel therapies for cancer [1]. The genus Pistacia is a member of the family Anacardiaceae, which is comprised of about 70 genera and over 600 species. Species of this genus (Pistacia khinjuk Stocks, Pistacia terebinthus L., Pistacia atlantica Desf., Pistacia vera L., and Pistacia lentiscus L.) are deciduous or evergreen resin-bearing trees and shrubs that grow up to 8–10 m height [5, 6]. Iran is the natural habitat of P. vera L., P. khinjuk Stocks., P. atlantica Desf., and P. atlantica [7, 8]. Pistacia, as a traditional herbal medicine, has been found to show different biological activities, such as anticancer and antioxidant properties, being able to improve glucose metabolism, reduce blood pressure, and control weight. Moreover, it can induce apoptosis [7]. This article aims to review Pistachios' potentials for influencing various apoptosis signaling pathways.

2. Cancer and apoptosis induction

Resistance to the induction of cell death is one of the signs of cancer. Thus, understanding essential mechanisms regulating different events of cell death, such as endoplasmic reticulum stress, apoptosis, necroptosis, and autophagy can help develop new agents for interfering with these pathways. Dysregulation of apoptosis allows the survival of neoplastic cells, even under conditions of oxidative stress and hypoxia, thereby noticeably contributing to pathogenesis [1]. Tumors can be formed as result of a series of genetic changes transforming normal cells to malignant ones [9]. In a study, Karimabad et al showed that a novel indole derivative triggered apoptosis and anti-cancer activity in NB4 cells by modulating the bax/bcl-2 (B-cell
lymphoma2) ratio [10]. Apoptosis can be induced as a non-surgical therapy for cancer by means of the agents that return apoptotic signaling pathways to normal patterns. It has been reported that apoptosis is associated with progression of tumors, hyperplasia, and formation of abnormal cells in an inverse manner [11]. Besides, it has been demonstrated that an abnormal cell can repress programmed cell death to become apoptosis-resistant through numerous mechanisms. Generally, apoptosis evasion mechanisms are categorized into three classes. These classes include imbalance between anti-apoptotic and pro-apoptotic proteins, a caspase activity decrease (cysteine-aspartic proteases, cysteine aspartases, or cysteine-dependent aspartate-directed proteases), and disruption of signaling pathways of death receptors. The ratio of the pro-apoptotic protein level to that of anti-apoptotic proteins is considered a fundamental factor in cell death modulation. Additionally, apoptosis modulation through down- or up-regulation of particular genes has been shown to play a significant role in carcinogenesis. Caspases are a type of these genes, which are generally categorized into two classes. The first class includes caspases 1, 4, 5, 13, and 14 that contribute to the cytokine processing during inflammation. The other class includes caspases 2, 3, 6, 7, 8, 9, and 10 that play the main role in apoptosis. Caspases 2, 8, 9, and 10 are known as "initiator caspases" because they initiate apoptosis; on the other side, "effector caspases" are comprised of caspases 3, 6, and 7 that interpose the cleavage of cellular components during the apoptosis process. In fact, caspases are involved in both initiation and execution of apoptosis. Consequently, disruption to the function or regulation of caspases can result in impaired apoptosis and carcinogenesis [13]. In their study, Mohammadizadeh et al confirmed that such disruption complicated activities of apoptosis from intrinsic and extrinsic apoptotic routes in malignant cells. However, because of the lack of considerable modifications to the bax/bcl-2 ratio in cells (L929) and the increase in the expression of caspase-8 and bid genes, this complication mainly activates apoptosis through the most effective extrinsic apoptotic pathways in regular cells [14]. There are various molecular mechanisms through which tumor cells suppress apoptosis. Decreased bax and increased bcl-2 in a tumor cell make it resistant to apoptosis [16, 17]. In addition, death receptors and their ligands have a substantial impact on external apoptosis signaling pathways. TNFR1, referred to as Fas, includes DR1, DR3, DR4, DR5, DR6, NGFR, and EDAR, being death receptors.
3. Pistachio applications in cancer cell treatment

The rising trend of cancers requires further studies to find more efficient therapies [18]. Radiotherapy, immunotherapy, chemotherapy, and transplantation of stem cells are the most popular methods used in treating tumors [18]. However, each of these modalities has its own limitations. For instance, chemotherapy and radiotherapy, being the most prevalent cancer treatment methods, have two main barriers, including side effects and disease recurrence [18]. Hence, the use of natural dietary elements, especially phytochemicals and medicinal plants, has received a lot of attention in recent decades [19, 20]. The highest rate of cytotoxicity of resins was observed against APL among 13 human cell line types [21]. Another study indicated antioxidant activities of green hull extracts of the Ahmadaghaei variety of pistachios [22]. Mastic gum extract delayed proliferation of colorectal cancers that progressed from colon tumor cells and xenografted into mice [23]. In this research, the hulls of ripe pistachios were extracted using methanol and ethanol, with their phenolic composition as well as antioxidant and cytoprotective activities determined. In both extracts, 20 compounds were identified, with the most abundant constituent having been gallic acid. The highest yields among all compounds were obtained using methanol as the extracting solvent. The results of this study highlighted the intense cytoprotective and anticancer activities of the components of pistachios [24]. Additionally, they have been reported to enhance expression of maspin (an inhibitor of mammary serine proteases in the cells of prostate cancer), thereby preventing growth of cell lines and blocking progression of the cell cycle [25, 26]. According to research, mastic oil of *P. lentiscus* significantly prevented proliferation of cancer cells in immune-competent mice with no signs of toxicity. This effect was exerted as a result of the induction of apoptosis, a reduction in neovascularization, and inhibition of chemokine expression [27]. In this regard, anticancer effects of various parts of *Pistacia* species have been examined. Accordingly, the extract of the *P. atlantica* sub. kurdica fruit exerted inhibitory effects on the growth of colon cancer cells, similar to those observed for doxorubicin [28]. Oleoresin extracted from *P. vera* exerted moderate cytotoxic effects on hepatocellular carcinoma, cervical tumor, breast tumor cells, and normal melanocytes [29]. In the same vein, the gum of *P. lentiscus* var. chia prevented growth of colorectal cancer cell lines and induced apoptosis [30]. Moreover, research showed pro-apoptotic and anti-proliferative effects of mastic oil on leukemia cell lines, which inhibited release of the vascular endothelial growth factor from these cells [31]. Furthermore, various
parts of P. lentiscus have been reported to show radical scavenging activities [21, 32, 33]. Cyanidin-3-O-glucoside, quercetin, epicatechin, luteolin, naringenin, and kaempferol are the main constituents of pistachio hull [34]. Concerning the extract of *P. terebinthus* leaves, an antioxidant capacity of approximately 12 times that of Butylated hydroxyanisole and ascorbic acid was observed [35]. Another study considered the mastic gum of the leaves and stem of *Pistacia lentiscus* as "conglomeration of effective anticancer drugs" and focused on different mechanisms of anticancer properties of its triterpenoids. This report considered anticancer properties for the resinous exudate and its major compounds [26]. The antioxidant property of *P. vera* (known as pistachio) nuts was observed to be similar to that of the synthetic antioxidant [36]. Interestingly, the antioxidant activity of the hydrophilic extract of *P. vera* nuts was significantly higher than that of its lipophilic extract [37]. The results obtained from four different assays indicated that the hull of *P. vera* had a stronger antioxidant activity than its kernel. That is because the hull has higher amounts of phenolic compounds acting as antioxidants [38]. According to research, other parts of *P. vera* show antioxidant properties as well [39]. Pistachio hull has been demonstrated to have antioxidant, enzyme-inhibitory, antimicrobial, and radical scavenging activities [40]. In addition, destructive effects of dietary kaempferol on cancers have been frequently reported [41]. In a research, anticancer properties of epicatechin have been reported [42]. The study conducted by Seifaddinipour *et al* demonstrated that the ethyl acetate extract of pistachio hull had no significant cytotoxic effects on normal fibroblast cells; however, it significantly affected all five tested human cancer cells, including HT-29, HCT-116, MCF-7, H23, and HepG2. Among these cell lines, HepG2 was the most resistant cell line, and MCF-7 was the most sensitive one [43].

Different *in vitro* and *in vivo* studies indicated anti-tumor effects of the flavonoid quercetin [42]. Besides, various *in vitro* antioxidant assays revealed that the leaves and fruit of *P. atlantica* showed antioxidant activities similar to or considerably higher than those of standard antioxidant compounds [44-46].

### 4. Pistachio targets during apoptosis induction in cancer cells

Table 1 shows the plant parts used as well as pharmacological activities of *Pistacia* from different regions. Growing evidence shows that pistachios exert their anticancer effects by influencing different apoptosis-related intrinsic and extrinsic pathways in cancerous cells (Fig.1).
Table 1 - Shows the plant parts used as well as pharmacological activities of Pistacia from different regions

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Plant part(s) used</th>
<th>Pharmacological activities</th>
<th>Assay</th>
<th>Model</th>
<th>Cell line Type of cancer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pistacia lentiscus</td>
<td>Japan</td>
<td>Resin</td>
<td>Cytotoxicity</td>
<td>MTT</td>
<td>In vitro</td>
<td>13 human cell line types (HSC-2, HSC-4, HSC-3, HepG2, T98G, U87MG, HGF, HPC, HPLF, HL-60, K-562, ML-1, KG-1)</td>
<td>[21]</td>
</tr>
<tr>
<td>Pistachia vera</td>
<td>Iran</td>
<td>Green hull extracts</td>
<td>Antioxidant, anti-microbial and antimutagenic</td>
<td>ABTS assay, DPPH assay, and β-carotene bleaching (BCB)</td>
<td>In vitro</td>
<td>Bacillus cereus</td>
<td>[22]</td>
</tr>
<tr>
<td>Lentiscus</td>
<td>Greece</td>
<td>Mastic gum</td>
<td>Induces p53- and p21-independent G1-phase arrest followed by apoptosis</td>
<td>Immunodeficient mice and tumor measurement</td>
<td>In vitro and in vivo</td>
<td>Colorectal cancers colon cancer/immunodeficiency, mouse model</td>
<td>[23]</td>
</tr>
<tr>
<td>Lentiscus</td>
<td>China</td>
<td>Mastic gum</td>
<td>Inhibits the ARE binding activity and increases the Sp1 binding activity in the Maspin promoter</td>
<td>RT-PCR and Western blotting</td>
<td>In vitro</td>
<td>Prostate cancer cells</td>
<td>[25]</td>
</tr>
<tr>
<td>Genus</td>
<td>Country</td>
<td>Material</td>
<td>Effect on Cells/Pathways</td>
<td>Method(s)</td>
<td>Route</td>
<td>Tumor Type</td>
<td>Reference</td>
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<tr>
<td>Lentiscus</td>
<td>China</td>
<td>Mastic gum</td>
<td>Blocks the PC-3 cell cycle in the G1 phase; Mastic gum decreases the p-AKT protein level and increases the IκBα protein level</td>
<td>MTT RT-PCR and western blotting</td>
<td>In vitro</td>
<td>Prostate cancer cells</td>
<td>[26]</td>
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<tr>
<td>Lentiscus</td>
<td>Greece</td>
<td>Mastic Oil</td>
<td>Blocks relevant signaling and transcription pathways</td>
<td>Immunohistochemistry and ELISA</td>
<td>In vivo</td>
<td>Lung carcinoma</td>
<td>[27]</td>
</tr>
<tr>
<td>Atlantica</td>
<td>Iran</td>
<td>Pericarp polyphenol-rich extract</td>
<td>Anti-proliferative, apoptosis induction, and cell cycle</td>
<td>MTT</td>
<td>In vitro</td>
<td>Human colon carcinoma, HT29 cells</td>
<td>[28]</td>
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<tr>
<td>Lentiscus</td>
<td>USA</td>
<td>Mastic gum</td>
<td>Apoptosis induction by CMG is not inhibited in the HCT116 cell.</td>
<td>CMG-treatment induces cell arrest at G1.</td>
<td>In vitro</td>
<td>Human colon cancer cells</td>
<td>[30]</td>
</tr>
<tr>
<td>Lentiscus</td>
<td>Greece</td>
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<td>Anti-proliferative and proapoptotic</td>
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<td>ELISA angiogenesis assays western blotting</td>
<td>In vitro</td>
<td>K562 Leukemia Cells</td>
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<td>Vera</td>
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<td>Pistachio nut</td>
<td>Antioxidant</td>
<td>TAA test</td>
<td>In vitro</td>
<td>Biological models</td>
<td>[37]</td>
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<tr>
<td>Atlantica</td>
<td>Iran</td>
<td>Flavonoid and flavonol content of the extract</td>
<td>Anticancer</td>
<td>MTT</td>
<td>In vitro</td>
<td>AGS, HeLa, and HDFs cells</td>
<td>[47]</td>
</tr>
<tr>
<td>Vera</td>
<td>Iran</td>
<td>Pistachio rosy hull (PRH)</td>
<td>Expression of both pro-apoptotic and anti-apoptotic genes associated with extrinsic and intrinsic apoptosis signaling pathways</td>
<td>MTT PCR array Flow cytometry</td>
<td>In vitro</td>
<td>HepG2</td>
<td>[48]</td>
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<tr>
<td>Vera</td>
<td>Iran</td>
<td>Extract of pericarp of pistachio fruit</td>
<td>Cytotoxic and apoptotic effects</td>
<td>MTT Realtime PCR</td>
<td>In vitro</td>
<td>HepG2</td>
<td>[53]</td>
</tr>
<tr>
<td>Vera</td>
<td>Iran</td>
<td></td>
<td>Cytotoxicity and apoptotic effects</td>
<td>MTT Realtime PCR</td>
<td>In vitro</td>
<td>MCF7</td>
<td>[54]</td>
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</tbody>
</table>
Fig. 1 - Effects of pistachios on apoptosis-related intrinsic and extrinsic pathways in cancer cells
This finding promises discovery of novel anticancer drugs for treating cancer by inducing apoptosis. Accordingly, the data obtained from the study of Hashemi et al on MCF-7 cell lines treated with three doses of F13b1/PV-EA indicated the dose-dependent effect of the compound on cell viability, which induced some apoptotic morphological changes to the cell lines [47]. Fathalizadeh et al examined the apoptotic effects of the aqueous extract of pistachio rosy hull (PRH) on HepG2 cells. Accordingly, they observed that the extract significantly reduced viability of the cell lines by inducing apoptosis. Besides, expression of many apoptosis-related intrinsic and extrinsic signaling pathways in cancerous cells was found out to alter in the cell lines treated with the PRH. The results of the polymerase chain reaction (PCR) array showed that 8 pro-apoptotic genes (CD27, lta, faslg, bag4, t, pycard, casp14, and casp6) were upregulated; in contrast, the remaining 9 pro-apoptotic genes (tnfrsf21, tnfrsf10a, bcl2l11, bax, casp3, casp4, casp7, casp10, and cradd) were downregulated. Besides, 5 anti-apoptotic genes (birc3, bcl2a1, xiap, cflar, and traf2) were downregulated. Downregulation of CFLAR (CASP8 and the FADD-like Apoptosis Regulator) is an interesting subject for cancer therapies because this cellular FLICE-like inhibitory protein (c-FLIP) plays an important role in apoptosis regulation [48, 49]. Research shows that p43-FLIP or the N-terminal fragment of the c-FLIP long isoform (c-FLIPL) interacts with TNF receptor-associated factor 2 (TRAF2), thereby activating NF-κB (the nuclear factor kappa-light-chain-enhancer of activated B cells) [49]. As a result, activation of NF-κB upregulates anti-apoptotic genes, which results in the survival of the cells [50]. Many cancers occur when the natural process of apoptosis is deregulated. Additionally, this dysregulation could cause resistance to chemotherapy among cancerous cells. Thus, one of the most efficient strategies for fighting cancer is to develop new drugs that regulate apoptotic molecules [51, 52]. In another study carried out by Harandi et al [53], the hydro-alcoholic extract of pistachio hull regulated the intrinsic apoptosis pathways in HepG2 cells by balancing the expression of bax/bcl-2 genes. The bax gene causes the release of cytochrome C and the subsequent apoptosis, while Bcl-2 protein blocks the cytochrome C channel. In their study, Ahmadirad et al examined anti-tumor effects of the hydro-alcoholic extract of wild pistachio leaves on breast cancer (MCF-7 cell line). Analysis results of the obtained data showed the IC50 values of 250 µg/mL and 400 µg/mL for cancerous MCF-7 and normal L929 cell lines, respectively, after treatment with the extract for 48 h. DNA fragmentation assays and morphological analysis
demonstrated apoptosis induction in both cell lines as a result of treatment at the concentration of IC50. Accordingly, upregulation of caspases -8 and -3, as well as bax and p53 reduced expression of bcl-2, which indicates that the extract induced apoptosis through extrinsic and intrinsic pathways in the MCF-7 cells. In fact, upregulation of p53, bax, and p21 as well as suppression of the expression of the bcl-2 gene induced apoptosis in the Hep G2 cell line. The P53 protein promotes expression of the bax gene, thereby directly activating transcription of the bax gene and inducing apoptosis [55]. Koyuncu et al. studied anticancer properties of different extracts of pistachio hull. Accordingly, they found out that the n-hexane fraction arrested the cell cycle at the G1 sub-phase and induced apoptosis through oxidative pathways in cancerous cell lines [56]. Seifaddinipour et al. evaluated cytotoxicity of different fractions of the ethyl acetate extract of pistachio hull using the 3-[4,5-dimethylthiazol-2-yI]-2,5-diphenyl tetrazolium bromide (MTT) assay. F13b1/PV-EA was found to be the most cytotoxic fraction, with the most abundant active compounds having been gallic acid and quercetin. Besides, the IC50 value of F13b1/PV-EA against MCF-7 cell lines was calculated to be 15.2± 1.35 µg/mL. This fraction increased expression of SOD, CAT, bax, as well as caspases 3 and 8 genes, yet it decreased that of bcl-2, according to the RT-PCR method. In vivo studies on cancer-induced mice revealed that F13b1/PV-EA inhibited development of the tumor [57].

5. Conclusion

The rising prevalence of malignant cancers worldwide, on the one hand, and various side effects of present treatments, on the other, have made it urgent to find novel treatments. Complementary and alternative therapeutic agents originated from herbal sources have attracted much attention due to their efficiency in interfering with oncogenic molecular signaling pathways. According to the results of the present research, phytochemicals of pistachios possess anticancer properties. This study focused on the role of the mentioned agents in apoptosis regulation. However, further studies are required to fully determine mechanisms of the anti-tumor activity of pistachios, especially their apoptotic activity.

Conflict of Interest

It is not applied to this study.

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