HAYDARPAŞA NUMUNE MEDICAL JOURNAL

DOI: 10.14744/hnhj.2021.46034
Haydarpasa Numune Med J 2023;63(2):115–119

hnhtipdergisi.com

ORIGINAL ARTICLE

Cytotoxic Effects of Punicalagin on U87, PANC-1, A549, and 22RV1 Cancer Cell Lines

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Abstract

Introduction: Cancer is an important public health problem worldwide and a disease that results in the highest number of deaths. Due to the existing drugs for cancer treatment, cumulative mutations, and drug interactions, treatment may fall insufficient. Hence, despite many new therapeutic approaches, there is still a need for more research to discover new molecules for treatment or for increasing drug efficacy. Regarding the development of new anticancer drugs, plant-derived agents stand out for their correlation with cancer. Flavonoids in our daily diet, together with other components like various vitamins, play a key role in the prevention of cancer.

Methods: The pure punicalagin flavonoid obtained from the pomegranate peel was studied in vitro on cancer cell lines such as U87, PANC-1, A549, 22RV1 and its cytotoxic effect was tested.

Results: It was observed that punicalagin showed cytotoxicity in all of the above-mentioned cancer cells, but was not cytotoxic in 293T human kidney epithelial cells.

Discussion and Conclusion: We hope that this study, which shows that Punicalagin has a selective cytotoxic effect, will shed light on other studies to investigate the detailed death mechanism of Punicalagin flavonoid and to be an alternative treatment to cancer treatments.

Keywords: Cancer; cytotoxicity; phenolic compounds; punicalagin.

Cancer is the most fatal disease in our age. It is defined as the progression of damaged cells through the cell cycle, which should have died due to the accumulation of functional disorders and mutations during the regulation of the cell cycle[1]. Basically, cancer is a genetic disease caused by the deterioration of information in DNA, the genetic material of cells. Examining cancer patients reveals abnormalities in gene expressions. Epigenetic changes that are not due to mutations are also very important factors[2,3]. Apoptosis, autophagy, and programmed necrosis, known as programmed cell death, occur when a cell is directed to death in the presence of a pathological condition in the cell within its own program. These three forms of programmed death decide the fate of the cell together[4].

The current drugs are insufficient in the treatment of cancer due to cumulative mutations. This creates a strong need for research to discover new molecules or increase drug efficacy in cancer treatment. Recent studies have contributed to the introduction of many potential chemotherapeutic agents to the market for developing new approaches against cancer and these agents have been successfully used in clinical applications. However, due to the side effects of existing chemotherapeutic agents and multidrug resistance, there is a need for new agents that can be used as alternatives or supplements to these agents in use. All research in this field focuses on revealing therapeutic agents that can exhibit selective cytotoxic and/or antiproliferative effects in cancer cells and not cause toxic responses in normal, healthy ones. Therefore, developing new alternative agents that can be considered both effective and safe are a key for cancer treatment. This is where plant extracts and metabolites isolated from plants stand out[5].

Regarding new anticancer drugs in terms of design, the correlation between plant-derived agents and cancer stands out

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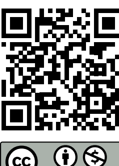
Submitted Date: 04.08.2021 **Revised Date:** 30.09.2021 **Accepted Date:** 18.10.2021



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and since each of these agents has a potential, researchers are attracted to this field. Plants and natural compounds obtained from plants are used for therapeutic purposes against many diseases. Numerous scientific studies have revealed that plants can be useful for treating many diseases affecting human life and quality of life, including infections, metabolic diseases, and cancer. Examining natural compounds obtained from plants pharmacologically, elucidating the mechanisms that provide such effects, and determining the component(s) responsible for these effects can guide research on this matter. Flavonoids are some of the natural compounds that have been tried in many clinical trials. Given the number of research on the anticancer effects of flavonoids, flavonoids seem to be potential anticancer agents[5-7].

Flavones are natural products with many derivatives and a common component in human nutrition. Reports indicate that they have a wide variety of biochemical and pharmacological effects, including antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory effects[8-10]. Pomegranate is a fruit in our daily diet that is consumed worldwide in fresh or dried extract form and is widely used for medicinal purposes. The peel of pomegranate, although it is a very valuable natural resource, is unfortunately often thrown away[11]. Pomegranate is particularly rich in phenolic compounds such as punicalagin and ellagic acid. Known as the component with the highest molecular weight, punicalagin is also the most common polyphenol in pomegranate. Punicalagin is a unique member of the ellagitannins family[12]. In the present research, we investigated whether punicalagin had a time or dose-dependent cytotoxic effect on various cancer cell lines.

Materials and Methods

Cells and Culture Conditions

PANC-1 (ATCC® CRL-1469™ human pancreatic cancer epithelial cell line), U87 (ATCC® HTB-14™, human brain cancer epithelial cell line), A549 (ATCC® CCL-185™ human non-small cell lung cancer cell line), 22RV-1 (ATCC® CRL-2505™ human prostate cancer epithelial cell line), and 293T (ATCC® CRL-1573™ human kidney epithelial cell line) cell lines were obtained from the Cancer Molecular Biology laboratory at Akdeniz University, Biology Department, opened in cell culture under appropriate conditions, grown in an RPMI 1640 medium, and enough passages were obtained to reach sufficient numbers for the experiment. All cells were incubated in a 5% CO_2 atmosphere at 37°C. The cells were lifted using a mixture of 0.25% trypsin, 0.03% EDTA, as recommended by ATCC, and passaged at a ratio of 1:2 or 1:3. Unused cells were stored in environments of 95% medium and 5% DMSO-containing freezing medium at –80°C.

Applying Punicalagin Flavonoid to Cells

Punicalagin flavonoid was obtained in pure form from TransMIT (PlantMetaChem- p=063), dissolved in serum-free medium, and stocked in RPMI medium as 2 mg/mL stocks. Cells were seeded into small petri dishes, removed by trypsinization when the dishes were 80–90% full, and seeded into sterile 96-well plates at 1×10^4 cells/well. The media were removed after 24 h, the drugs were added to media containing 1% serum by reducing the doses in half (200–25 µg/mL) by serial dilution, with a maximum dose of 200 µg/mL. The incubation period was determined as 24 h. This way, we tried to determine the time and dose-dependent cytotoxic effects of punicalagin on cell lines. Cell viability was determined using a WST-1 cell proliferation kit.

Wst-1 Cell Proliferation Test

(CAYMAN Kat No:1000883) The cytotoxic effect of punicalagin was investigated using a WST-1 cell proliferation kit. The WST-1 test is based on the principle that the metabolic activity in living cells breaks down WST-1 through the mitochondrial dehydrogenase enzyme, forming soluble formazan salts. In the test, the cells were seeded into small petri dishes, removed by trypsinization when the dishes were 80–90% full, and seeded into sterile 96-well plates at 1×10^4 cells/well. The media were removed after 24 h and all doses were prepared in medium containing 1% fetal bovine serum with a maximum dose of 200 µg/mL (200–25 µg/mL) added to the wells. After the 24-h incubation period, the media were withdrawn, 90 µL of WST-1 solution was added to 10 µL of serum-free media, and allowed to incubate for an average of 4 h. After incubation, the absorbance values of the plates were measured using a spectrophotometer at a wavelength of 450 nm.

Statistical Analysis

Differences in cytotoxicity findings between control and other groups were evaluated using one-way ANOVA test and then the Dunnett's multiple comparison test on the Graph-Pad InStat statistical software. All data are plotted as mean±SEM using the Sigma Plot 10.0 software.

Results

Cytotoxic Effect of Punicalagin on U87, PANC-1, A549, 22RV1, and 293T Cell Lines by WST-1 Test

The cytotoxic effect of punicalagin flavonoid on cell lines U87, PANC-1, A549, 22RV1, and 293T was tested at doses of 200, 100, 50, 25 µg/mL in 24-h incubation periods. Each experiment was repeated 4 times. In line with the findings, the effect of applying punicalagin to cell lines U87 (Fig. 1), PANC-1 (Fig. 2), A549 (Fig. 3), 22RV1 (Fig. 4), 293T (Fig. 5) in

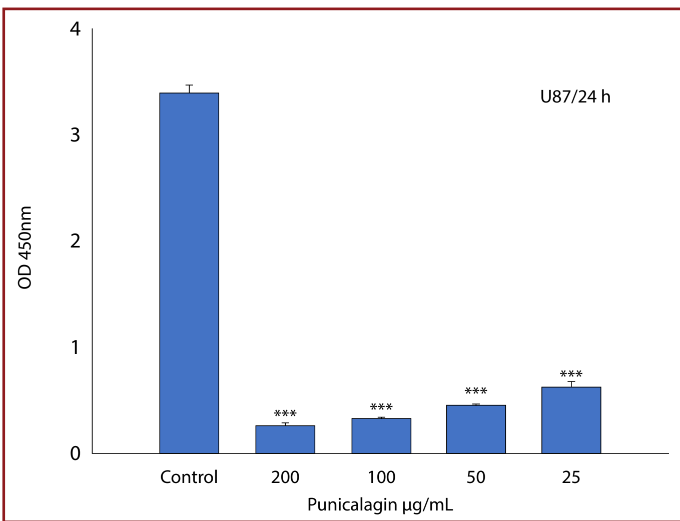

Figure 1. Effect of punicalagin on cell viability in U87 between 200 and 25 µg/mL over 24-h incubation (***p<0.001).

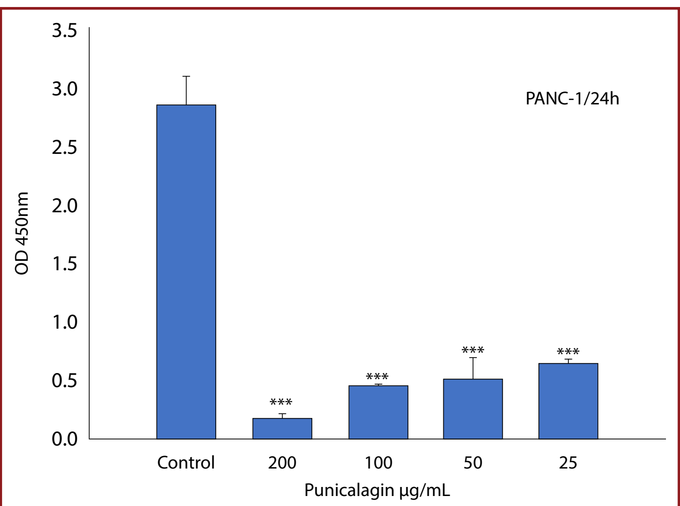

Figure 2. Effect of punicalagin on cell viability in PANC-1 between 200-25 µg/mL over 24-hour incubation (***, p<0.001).

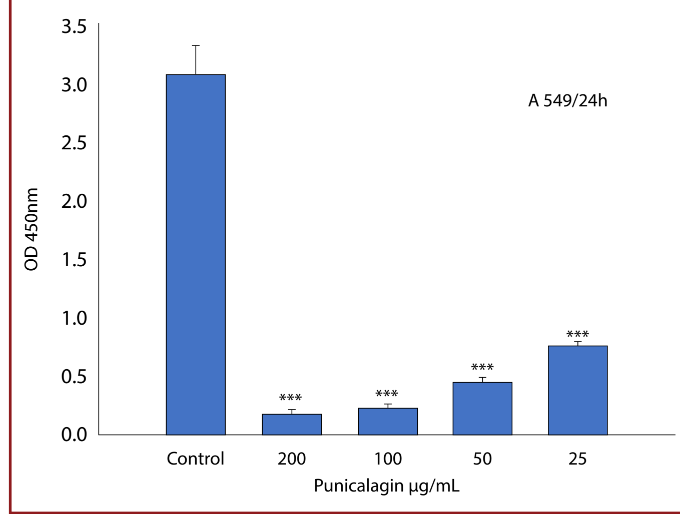

Figure 3. Effect of punicalagin on cell viability in A549 between 200-25 µg/mL over 24-hour incubation (***, p<0.001).

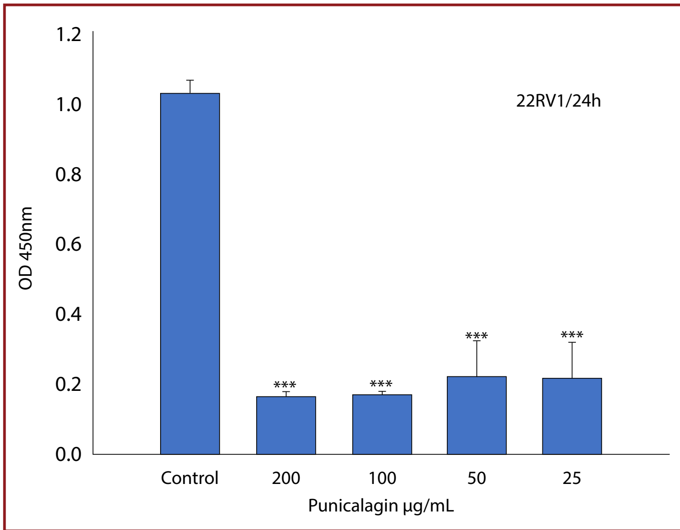

Figure 4. Effect of punicalagin on cell viability in 22RV1 between 200-25 µg/mL over 24-hour incubation (***, p<0.001).

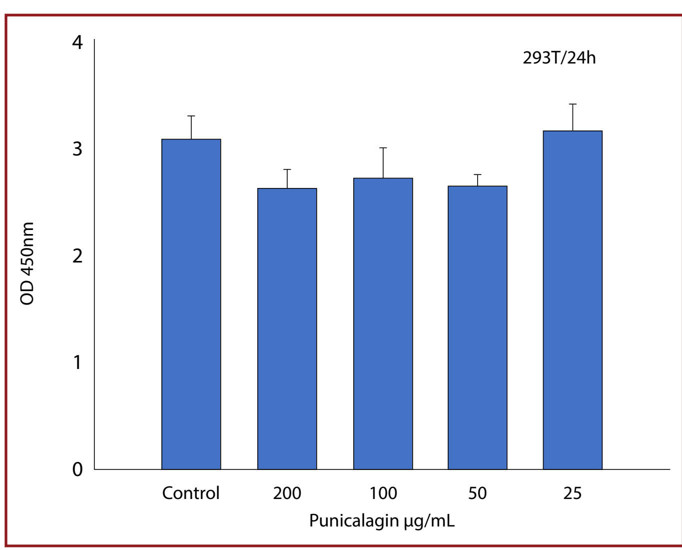

Figure 5. Effect of punicalagin on cell viability in 293T between 200-25 µg/mL over 24-hour incubation.

the range of 200–25 µg/mL on cell viability was evaluated and plotted using the SigmaPlot statistics software.

Conclusion

Despite great efforts in the fight against cancer for half a century, inadequate classical cancer treatments have been supported with new therapeutic approaches. All research in this field aims to develop new therapeutic agents that can exhibit selective cytotoxic and/or antiproliferative effects in cancer cells, without causing toxic responses in normal, healthy cells. Hence, developing new agents that are both effective and safe are extremely crucial for cancer treatment. This is where plant-based extracts and metabolites isolated from plants stand out[5]. Considering these

metabolites, the first one that comes to mind is flavonoids, always in our lives through nutrition.

The correlation between cancer and nutrition is supported by national and international epidemiological studies. Evidence shows that a diet rich in fruits and vegetables can reduce the risk of common types of cancer; both basic and clinical studies have shown benefits of certain classes of phytochemicals[13].

Flavonoids are thermostable phytochemicals, commonly found in nature as free aglycones or glycosidic derivatives. In terms of structure, they are divided into six groups as anthocyanidins, flavonols, flavones, flavanones, flavanols, and isoflavones. Differences in structure create the potential anticancer effects of flavonoids. Flavonoids have stood out among natural agents in recent anticancer research, because they suppress the cell cycle, induce apoptosis, inhibit mitotic spindle formation, and inhibit angiogenesis. Flavonoids are highly valuable biological components and are consumed in significant amounts in our daily diets. Numerous studies indicate that flavonoids have a wide variety of biological effects, including antiallergic, anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic effects[9,14-19].

Dietary flavonoids, along with other components like various vitamins, play a key role in the prevention of cancer. The effects of flavonoids on cell signal transduction pathways associated with reactive oxygen derivatives, cellular proliferation, apoptosis, and angiogenesis make them highly interesting for research at the molecular level. The mechanism of effect of flavonoids on cancer cells continues to be investigated. Studies suggesting dietary flavonoids as a cancer preventive approach are promising[5,15,20-22].

Widely consumed as a part of human life for years, pomegranate (Punica granatum L.) is a valuable fruit that contains important phytochemicals and is also used for therapeutic purposes. Pomegranate shows anticarcinogenic effect on various cancer cell types. Research has shown pomegranate extracts to exhibit anti-proliferative, pro-apoptotic, anti-invasive, and/or anti-inflammatory effects on cancer cell lines[23-27]. Punicalagin is a medicinal bioactive ingredient with high molecular weight obtained from the peel of pomegranate, also abundant in pomegranate seeds and juice. A water-soluble phenolic compound, punicalagin has been shown to exhibit anti-oxidant, antitumor, antiatherosclerosis, anti-inflammatory, anti-viral, anti-fungal, and anti-bacterial effects, directly or indirectly. Besides, numerous studies show that punicalagin has anticancer effects. It exhibits these effects by suppressing the development of tumor cells or through anti-proliferative and apoptotic effects. Its positive effects on health and non-toxic nature make it multifunctional[11].

Seeraam et al.[28] evaluated punicalagin, ellagic acid, and total pomegranate tannins for their antiproliferative effects and found them to be effective on human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620), and prostate (RWPE-1, 22RV1) cancer cells. They also reported that it exhibited apoptotic effects, particularly on HT-29 and HCT116 colon cancer cell lines.

Here, punicalagin flavonoid in pomegranate peel was tested for its cytotoxic effects on four different cancer cell lines at doses of 200, 100, 50, and 25 µg/mL over a 24-h incubation period. Punicalagin had a statistically high cytotoxic effect on cancer cell lines PANC-1, 22RV-1, U87, and A549 at all doses and no statistically significant cytotoxic effect on 293T, which was used as the control group. This indicates that punicalagin is very important and promising for selective cytotoxicity. Based on our findings, we hope that further research on punicalagin will shed more light on the mechanism of death in these cells and on the potential of punicalagin as an alternative cancer treatment.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: A.C.K., E.A.; Design: A.C.K., E.A.; Supervision: A.C.K., E.A.; Fundings: A.C.K.; Materials: A.C.K.; Data Collection or Processing: A.C.K., E.A.; Analysis or Interpretation: A.C.K., E.A.; Literature Search: A.C.K., E.A.; Writing: A.C.K.; Critical Review: A.C.K., E.A.

Conflict of Interest: None declared.

Financial Disclosure: The authors declared that this study received no financial support.

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