

Apoptotic Effect of *Haplophyllum Buxbaumii* Extract on Prostate Cancer

Haplophyllum Buxbaumii Ekstresinin Prostat Kanseri Üzerindeki Apoptotik Etkisi

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ABSTRACT

Prostate cancer is a common type of cancer among men. Recently, studies on new anticancer agents of herbal origin in the treatment of cancer have increased. *Haplophyllum buxbaumii* (HP) is a perennial herbaceous plant. Studies have reported that *Haplophyllum* subspecies has antioxidant properties. And the anticancer effect of HP is unknown. This study aims to reveal the apoptotic effect HP on prostate cancer. The cytotoxic effect of HP was evaluated by the MTT method. In addition, apoptotic pathway mediators were analyzed by ELISA method. HP extract application reduced the viability of prostate cancer and activated the apoptotic mediators while decreasing anti-apoptotic mediators. Our study showed that HP extract has an antiproliferative and apoptotic effect on prostate cancer.

Keywords: Haplophyllum Buxbaumii, Apoptosis, Cytotoxicity, Prostate Cancer

ÖZ

Prostat kanseri, erkekler arasında yaygın bir kanser türüdür. Son zamanlarda, kanser tedavisinde bitkisel kökenli yeni antikanser ajanlar üzerine yapılan çalışmalar artmıştır. *Haplophyllum buxbaumii* (HP) çok yıllık otsu bir bitkidir. Çalışmalar, *Haplophyllum* alt türlerinin antioksidan özelliklere sahip olduğunu bildirmiştir. HP'nin antikanser etkisi bilinmemektedir. Bu çalışma, HP'nin prostat kanseri üzerindeki apoptotik etkisini ortaya çıkarmayı amaçlamaktadır. HP'nin sitotoksik etkisi MTT yöntemi ile değerlendirilmiştir. Ayrıca apoptotik yol aracı medyatörleri ELISA yöntemi ile analiz edilmiştir. HP ekstresi uygulaması, prostat kanserinin canlılığını azaltıp apoptotik medyatörleri aktive ederken aynı zamanda antiapoptotik medyatörleri azaltmıştır. Çalışmamız, HP ekstresinin prostat kanseri üzerinde antiproliferatif ve apoptotik bir etkiye sahip olduğunu göstermiştir.

Anahtar Kelimeler: Haplophyllum Buxbaumii, Apoptozis, Sitotoksosite, Prostat Kanseri

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INTRODUCTION

According to data from developed countries, prostate cancer is the second most frequently diagnosed type of cancer and the fourth most common cause of death. The incidence of prostate cancer in men over the age of fifty has been found to be 30% with autopsy studies.¹ Many studies have shown that most chemotherapeutic drugs are highly cytotoxic and non-specific as they target all proliferative cells. This leads to a poor prognosis for cancer patients.² Recently, due to the cytotoxic effects of chemotherapeutics, the trend towards naturally derived anticancer drugs has

increased. In this context, it is aimed to investigate and develop new anti-cancer agents that selectively target cancer cells.

HP is a perennial herbaceous plant. HP can grow in the steppe, barren and fallow land or cultivated land. The main distribution area of the HP plant is the Syrian Desert and Iran. It is also seen around Şanlıurfa in Turkey. Some studies have shown that the subspecies of HP is protective against oxidative stress.³ There is limited information about this plant in the literature. This study aims to reveal the apoptotic effect HP on prostate cancer.

MATERIALS AND METHODS

The in vivo part of this study has been made in the laboratory of Çukurova University, Faculty of Medicine, Department of Pharmacology. The materials which used for this study are from the laboratory. The chemicals used are listed below.

The above-the-ground parts of the HP plant used in the study were dried in the oven. After the dried plant samples were powdered, methanol: water prepared with a ratio of 500 g (1:1) was incubated in a 40°C overnight shaker and thoroughly homogenized for 15 minutes with an ultrasound homogenizer. Alcohol solvents were blown with a rotary evaporator so that they would not exceed 40°C, filtered with Whatman filter paper, and the extracts were obtained through powdering in the lyophilizer.

Cell Culture and Homogenization

Human prostate adenocarcinoma (PC3) cancer cell line has been obtained from American Type Culture Collection (ATCC, US). PC-3 was proliferated in modified F-12K medium by adding 2 mM L-Glutamine, 10% heat inactivated fetal bovine serum (FBS), 1% streptomycin and penicillin with a 5% CO₂ supply at 37 °C.

The cells (5×10⁴ cells/cm²) were treated with 20 µg/ml HP extract for 48 h.

Then, the cells were washed with PBS. The cells were then harvested. They were lysed in RIPA buffer with an ultrasonic homogenizer. After the homogenization, the homogenates were centrifugated at 15000 rpm and +4°C for 20 min and the supernatants were taken and pellets were discarded.

Cell Viability Assay

The cell viability of PC3 was analyzed by the MTT method, which was described above.⁴ The cells were treated with a dose of %2-40µg/ml of HP and were examined after 48h. After the treatment with HP, the results were obtained

Total Protein Quantification

Bradford method was used to determine the total protein in the homogenates of the cells.⁴

Determination of Cleaved Caspase-3, Bax and Bcl-2

ELISA test was used to determine cleaved caspase-3, Bax and Bcl-2 expression. Assays protocol was performed according to its manufacturer's instructions.^{5,6}

Ethical Aspect of Research

In this study, an ethics committee application is not required, as the study

was conducted on cell culture. This study has been made in the laboratory of Çukurova University, Faculty of Medicine, Department of Pharmacology. Institutional permission was obtained for this study on April 12, 2020.

Data Analysis

Unpaired Student's t test was used to compare the differences among the groups. The data is shown as \pm SEM. A value of $p < 0.05$ was considered statistically significant.

Limitation of Research

Conducting the study on cell culture is seen as a research limitation.

Conflict of Interest

No conflict of interest was declared by the authors.

Financial Disclosure

The authors declared that this study received no financial support.

RESULTS AND DISCUSSION

The PC3 cells were treated with 20g/ml of HP extract for 48 h to reveal its anti-proliferative effect. HP treatment significantly increased expression of the Bax (Fig 1a) and cleaved-caspase 3 (Fig 1b), which are proapoptotic mediators in PC3 cells ($p < 0.05$). HP treatment also

significantly reduced levels of the anti-apoptotic BCL-2 (Fig 1c) protein in PC3 cells ($p < 0.05$).

48 hour HP treatment reduced the cell viability of the PC3 cell in various doses (Fig 1d).

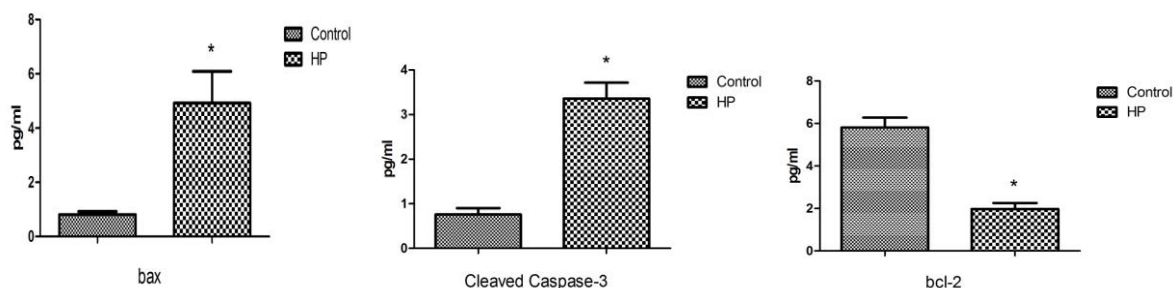


Figure 1.a. Effect of HP on Bax Expression **Figure 1.b.** Effect of HP on Caspase-3 Activity **Figure 1.c.** Effect of HP on BCL-2 Expression

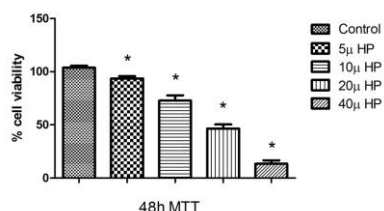


Figure 1.d. Effect of Extract Treatment on Cell Viability (MTT).
* Difference Versus Control Group is Significant with $p < 0.05$.

In this study, we revealed the anticancer efficacy of the HP plant, the

medical use of which is not covered much in the literature. We found that the HP

reduced the viability of PC3 cancer cells at various doses while inducing the apoptotic pathway.

In recent years, there has been a significant increase in the interest in naturally obtained compounds in the treatment of cancer. Some plants that are used as food, vegetables, fruits or spices are rich in sources of bioactive phytochemicals. Recent studies offer phytochemicals as an option for cancer prevention and treatment.⁷ Drugs obtained from plants are used in the treatment of various diseases worldwide and plants and their derivatives have been used in medical treatments since ancient times. Recently investigations on the properties of some plant-based compounds revealed that they affect carcinogenic activity in various ways.⁸ Various plants of cultural origins are used by physicians to cure many diseases. The use of plant-derived products in cancer treatment may not be toxic to normal cells and is better tolerated. Therefore, it attracts the attention of modern pharmaceutical researchers.⁹

At least 250.000 species have been examined but only 10 percent of these plants have been researched for pharmacological applications. Phytochemicals and their derivatives in roots, leaves, flowers, stems, and bark affect various functions in human metabolic systems pharmacologically.^{7,10,11}

As a result of newly conducted drug production and innovation studies, the interest in phytotherapy drugs with fewer side effects than chemotherapy has increased. The biggest disadvantage of the drugs used in cancer treatment is that they are not specific and their side effects are quite high. Therefore, it is not possible to talk about the application of the radical treatment method, which presents the importance of the need for cancer drugs without side effects. In our study, we revealed the anticancer efficacy of the HP plant, the medical use of which is not covered much in the literature.

According to the results of our study, HP pc3 reduced dose-dependent cell viability in cancer cells. The most important feature expected from substances to be used as a medicine in pharmacology is that they are dose dependent. This is an advantage for designing an anti-cancer drug from the HP plant. It also induced the apoptotic pathway directly. There are many substances in the content of HP and it is not known which substance and by which mechanism this apoptotic effect occurs. In addition, there is not much literature information about this plant and it has the potential to attract the attention of researchers. This is the first study in which HP plant extract is tested on human prostate adenocarcinoma.

CONCLUSION AND RECOMMENDATIONS

Based on the data obtained, it was determined that this plant had anticancer activity. When we analyze the data obtained in this study, we believe that HP

plant extracts will shed light on innovative and unique studies in the treatment of prostate cancer and other cancers.

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