ORIGINAL ARTICLE

Apoptotic effects of *Acorus calamus* extract on prostate cancer LNCaP cells

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Abstract

Acorus calamus is a plant that is widely used in Far Eastern and Asian countries and has anti-inflammatory, anti-oxidant, anti-microbial, and anti-cancer effects. Prostate cancer is a sort of cancer that is frequently diagnosed and has a high mortality rate in men. Only a limited number of studies are available showing the effects of *Acorus calamus* on prostate cancer. To assess the effects of *Acorus calamus* extract on caspase and anti-apoptotic and pro-apoptotic markers that play a role in the apoptotic process of LNCaP cells in prostate cancer by conducting an in vitro study. LNCaP cells were incubated for 24 and 48 hours and treated with different concentrations of an ethanolic extract of *Acorus calamus* ranging from 250 to 700 µg/ml. Caspase-3, -8, and -9, Bcl-2, Bax, APAF-1, Bcl-XL, and p53 levels were measured using the ELISA method. Quantitative gene expression analyzes of Bcl-2 and Bax were performed using real-time reverse transcription–polymerase chain reaction. The Mann-Whitney U and Tukey tests were used to analyse differences between groups. $p<0.05$ was considered statistically significant. Caspase-3 and -8 and APAF-1 levels were found to be significantly higher in the 48th-hour application of 700 µg/ml of *Acorus calamus* extract than in the control group ($p<0.05$, $p<0.001$, and $p<0.001$, respectively). Bcl-2 was significantly lower and Bax/Bcl-2 expression ratio was significantly higher at all doses for 24 and 48 hours compared to the control group ($p<0.001$ for all). Although, caspase-9, Bcl-XL, and p53 were higher in experimental groups than controls, no significant difference was found. This study supported the time- and dose-dependent anti-cancer effects of *Acorus calamus* on LNCaP cells of the prostate cancer type. Further preclinical and clinical studies are requisite to support our findings.

Keywords: Acorus calamus, prostate cancer cells, LNCaP, apoptosis, caspases

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Introduction

Prostate cancer is the second most frequently type of solid tumor in men in terms of both frequency and cancer-related mortality [1]. Prostate cancer incidence is increasing under the age of 50 years, and the treatment of cases with refractory metastatic spread is especially difficult [2]. Radiotherapy and chemotherapy have serious side effects, and the cost of prostate cancer treatment is high in terms of health expenditures [3,4].

Due to drug resistance, drug toxicity, and high relapse rates, there has recently been an increasing need for natural substances and phytochemical compounds to determine safer sources to be used in the treatment of cancer [5]. In addition, with the positive results obtained in the treatment of neurodegeneration, diabetes, and cardiovascular diseases, interest in natural/herbal resources in cancer treatment is increasing, and the positive effects of phytochemicals such as galantamine, rivastigmine, and resveratrol have been demonstrated [6]. Phytochemicals isolated from plants and their extracts show activity against prostate cancer. They are considered appropriate for use in combination with existing chemotherapy, albeit not alone [7].

Randomized placebo-controlled clinical trials have been conducted with curcumin, lycopene, isoflavone, and sulforaphane in the treatment of prostate cancer, with flavone, berberine, capsaicin, silibinin, and noscapine being among the phytochemicals whose preclinical research continues [8,9]. Many of these compounds target cancer-specific pathways, such as growth-metabolic (PI3K/AKT/mTOR/CDK and AR), angiogenetic (vascular endothelial growth factor), proinflammatory (NF-kB), tumor suppressive (p53/Rb), and invasive and metastatic (WNT/beta catenin) pathways [10].

Acorus calamus (AC) is a plant native to India and used in Chinese medicine for the treatment of gastrointestinal, respiratory, neurological, kidney, liver, hypertension, metabolic disorders such as diabetes, and obesity. AC's main components are monoterpenes, sesquiterpenes, quinin, flavonoids, phenylpropanoids, and the volatile compounds alpha- and beta (β)-asarone.

AC also has good total nitric oxide and nitrite reducing activity. [11]. The anti-inflammatory, neuroprotective, anti-fungal, anti-oxidant, anti-microbial, anti-allergic, anti-ulcer, and cardioprotective effects of AC, as well as its anti-tumor and tumor protective properties, have been confirmed by in vivo and in vitro preclinical pharmacological studies [12]. It has been indicated that β-asarone, one of the main components of AC, can stimulate apoptosis in and reduce the invasive capability of cancerous cells and have antiproliferative effects in glioblastoma and gastrointestinal system cancers [13-15]. β-asarone nitro derivatives in prostate cancer (PC-3 cells), synovial cancer (SW982 cells), cervical cancer (HeLa cells), neuroblastoma (IMR-32 cells), and breast cancer (MCF-7 cells) have been shown to cause a decrease in the viability of the cancer cells [16].

In previous in vitro research, we showed the dose-response relationship of the ethanolic extract of AC with prostate cancer LNCaP cells and reported its anticancer and anti-angiogenic properties [17]. In the current study, we purposed to evaluate the effect of the AC ethanolic extract on caspases [caspase-3, -8, and -9 and apoptotic protease activating factor-1 (APAF-1)], which get involved in the apoptotic process in prostate cancer LNCaP cells, and on Bcl-2-associated X-protein (Bax), Bcl-2, Bcl-XL, and p53, which are both anti- and pro-apoptotic markers. Studies showing the effects of AC on prostate cancer are limited in literature. With this study, it is planned to support the results we found in the previous study and to present the results of their combined use with chemotherapeutic agents with in vitro studies after this study.

Materials and Methods

Cell Line and Culture Conditions

The LNCaP cell was provided from the Department of Physiology of Yeditepe University (Istanbul, Türkiye). Cells cultured in Roswell Park Memorial Institute 1640 Medium fortified with 100 U/mL penicillin G, 10% fetal bovine serum, and 100 mg/mL streptomycin. Cells maintained at 37 ^{0}C in a humidified incubator under atmospheric conditions of 5% carbon dioxide and 95% air.

Preparation of the Ethanolic Extract of AC

Whole freshly AC was obtained from the local herbal shop. The plant was processed at the Medicinal Plants, Drugs, and Scientific Research Center of Anadolu University. The entire plant was separated into smithereens and dried in the dark. The desiccated herb was ground into powder using a hand-held grinder and sifted. The crude powder was extracted with 70% ethanol in a Soxhlet apparatus for 12 h. Vacuum evaporation was used to concentrate the ethanol extract. The yield of the ethanolic extract was found to be 28.29%. Before application the ethanolic extract was freshly dissolved in dimethyl sulfoxide.

Apoptosis Assay: Caspase-3, -8, and -9, APAF-1, Bax, Bcl -2, Bcl-XL, and p53

Caspase-3, -8, and -9, Bcl-2, and p53 were assayed using their specific Invitrogen Human ELISA Kits (Thermo Fisher Scientific, USA). APAF-1, Bax, and Bcl-XL were assayed using their respective Sunred Human ELISA Kits (Shanghai Sunred Biological Technology Co., Ltd, China). The cells have been seeded at 1×10^4 cells per well into culture medium including different concentrations (0, 250, 500, and 700 μg/ml) of the ethanolic extract of AC. For the determine apoptotic effects the cells were incubated for 24 and 48 hours. In summary, following treatment, the cells underwent two washes with icy cold phosphate-buffered saline solution. They were then lysed using 500 μl of ice-cold lysis buffer, scraped, and sonicated while on ice. Then, the cell lysate was centrifuged at 4 0C for 10 minutes, and before analysis the supernatant was stored at -80 ^{0}C. Each well of the ELISA plate, 100 microliters of soluble fraction were used. The ELISA kits were performed in accordance with the manufacturer's instructions and absorbance value was measured at a wavelength of 450 nm.

Bax and Bcl-2 mRNA Amplification by Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from cultured LNCaP cells using the RNA Isolation Kit according to the suppliers' directions (A.B.T.™ RNA Purification Kit). After measuring absorbance at 260 nm on a UV spectrophotometer, RNA concentration and purity was calculated and then stored at -80 ^{0}C. The cDNA Kit was used for first-strand cDNA synthesis (VitaScript™ FirstStrand cDNA Synthesis Kit). Polymerase chain reaction (PCR) amplification was performed using the synthesised cDNA as a template. Real Time PCR was carried out (Connect Real Time PCR Detection System) with amplification of human beta actin mRNA as a housekeeping standard. The forward primer for Bax was 5'-GCCGAAATGTTTGCTGACG-3'. The reverse primer was 5'-CGCCACGGTGCTCTCC-3'. The forward primer for Bcl-2 was 5'-ACTTTGCCGAGATGTCCAGC-3' and the reverse primer was 5'-ATCCCAGCCTCCGTTATCCT-3'. The following conditions were applied: initial denaturation at 95 ^{0}C for 5 min, followed by 40 cycles of denaturation at 95 ^{0}C for 15 s, annealing at 60 ^{0}C for 30 s, and extension at 72 ^{0}C for 30 s. Automated threshold analysis was used to determine cycle thresholds. Melting curve analysis was used to confirm primer quality. Relative quantification of the gene expression was carried out by using a standard curve generated from serially diluted of control mRNA or real-time PCR amplicons. All trials were performed three times. Bax and Bcl-2 levels were standardized to beta actin to account for loading differences.

Study Groups

In our previous study, cells were treated with different concentrations (0, 250, 500, 750, 1000 and 1250 μg/mL) of AC for 24 h and 48 h. After 48 h, the XTT measurement was performed. This assay is commonly used to measure cell viability, and the percentage of cell viability was calculated using a spectrophotometer. The IC_{50} value is determined as the concentration that reduces cell viability by 50%. After 48 hours, the XTT measurement was performed, and the IC_{50} value of the AC concentration was calculated to be 732.76 μg/ml [17]. Accordingly, three concentrations (250, 500, and 700 μg/ml) were chosen to be used in experiments to find out the effects of AC on LNCaP cells. The following groups were formed by applying the specified extract concentrations to the LNCaP cells separately for 24 and 48 hours to determine the effects of the ethanolic extract on apoptotic markers.

Group 1: Control group, to which only the medium was applied (n = 6)

Group 2: 250 µg/ml extract, (24 and 48 hours, n = 6)

Group 3: 500 µg/ml extract, (24 and 48 hours, n = 6)

Group 4: 700 µg/ml extract, (24 and 48 hours, n = 6)

Statistical Analysis

All data were obtained from six independent experiments in each group and presented as mean ± standard deviation. The Shapiro-Wilk test was used to test the normality of the distribution of the continuous variables. Tukey test was used for normal distributed variables and Mann-Whitney U test was used for non-normal distributed variables. All data were analysed using the statistical programme SPSS v. 24 (SPSS Inc., USA), and $p<0.05$ considered to be statistically significant.

Results

Table 1 and **Figure 1** present the measurements of apoptotic markers in the applications of 250, 500, and 700 µg/ml of AC extract to LNCaP cells for 24 and 48 hours and their comparison between the experimental groups.

At the 24th hour of AC application, no significant difference was found between the groups in terms of caspase levels. At the 48th hour, however, the high-dose (700 µg/ml) application of AC resulted in significantly higher Caspase-3 levels compared to the control group ($p<0.05$). Caspase-8 levels increased as the AC dose increased at the 48th hour, but this increase was only significant at the highest dose (700 µg/ml) ($p<0.001$). The caspase-9 level was higher at the highest dose of AC compared to the control group, but it was not statistically significant. Similar to the caspase level, the APAF-1 level did not significantly differ between the groups at the 24th hour but was significantly higher at the 48th hour at the highest dose of AC ($p<0.001$).

When the anti-apoptotic marker Bcl-2 was examined, its levels were found to be statistically significantly lower at all doses of AC at both 24 and 48 hours compared to the control group ($p<0.001$). Regarding the other anti-apoptotic marker, Bcl-XL, there was no significant difference between groups.

While there was no significant difference in the levels of the pro-apoptotic marker Bax at the 24th hour, it was determined to be significantly higher at the 500 and 700 µg/ml doses of AC at the 48th hour compared to the control group ($p<0.05$ and $p<0.001$, respectively). Similar to Bax, another pro-apoptotic marker, p53, was found to have higher levels at all doses of AC at both the 24th and 48th hours compared to the control group, but the differences were not statistically significant.

When the Bax/Bcl-2 expression ratio was examined, there was a significant increase at low, medium, and high doses of AC at the 24th and 48th hours compared to the control group ($p<0.001$ for all, **Figure 2**).

Discussion

In the treatment of cancer, medicinal plants are used. Using plants to treat cancer continue to be investigated. In this study, the anticancer features of the ethanolic extract of the AC plant on prostate cancer LNCaP cells were investigated. It was found that AC had a greater effect on markers regulating apoptosis, especially in longer-term (48-hour) and high-dose applications.

One of the mechanisms of action of cytotoxic drugs on cancer cells is apoptosis. It is a caspase-dependent, programmed active cell death that occurs in a well-organized, non-inflammatory intrinsic and/or extrinsic pathway and takes a consequential role in the development and homeostasis of multicellular organisms [18]. In the current study, the caspase-3 and caspase-8 levels of the LNCaP cells exposed to high-dose AC extract for 48 hours significantly increased compared to the control group. Caspase-8 is an initiator caspase that is involved in the extrinsic pathway [19]. Caspase-3, on the other hand, belongs to the executioner caspase family. In recent years, in vitro studies on the effects of plant extracts on LNCaP cells have indicate promising developments [20]. In a study by Zhou et al., the caspase-3 level was found to increase in LNCaP cells treated with *Linum usitatissimum* (flax) [21]. In another study, Nikahd et al. reported an increase in caspase-3 activity in LNCaP cells, to which the extract of *Daphne pontica* was applied using the colorimetric assay method [22]. All

these studies emphasize the importance of the apoptosis pathway and the role of caspases in this pathway in the cancer treatment mechanism and support our findings.

Caspase-9 and APAF-1 play roles in the intrinsic pathway activated by mitochondrial damage in apoptosis [23] and form the apoptosome complex. In the case of intracellular stress, such as hypoxia and drug-induced DNA damage, release of cytochrome c into the cytosol and binds to APAF-1, promoting the formation of the apoptosome [18]. APAF-1 has been shown to play a role in the development of the brain, apoptosis resistance in neurons and cardiomyocytes, and the pathogenesis of neurodegenerative diseases [24-26]. It has been reported to be involved in the therapeutic mechanisms of MCF-7 cells in breast cancer, oral cancer cells, in lung cancer, and TP53-regulated inhibitor of apoptosis-1 cells in

Table 1. Apoptotic marker measurements of experimental groups involving the application of different concentrations of *Acorus calamus* extract to LNCaP cells for 24 and 48 hours.

| Experimental Groups	Mean, ng/ml (SD)							
	Caspase-3	Caspase-8	Caspase-9	APAF-1	Bcl-2	Bcl-XL	Bax	p53
Control								
24 hours	6.56 ± 0.65	2.38 ± 0.04	10.97 ± 1.18	4.64 ± 0.42	26.81 ± 6.63	7.37 ± 1.22	1.40 ± 0.14	22.43 ± 4.16
48 hours	6.79 ± 2.43	2.37 ± 0.02	11.90 ± 2.20	4.33 ± 0.47	42.53 ± 3.52	6.78 ± 0.24	1.26 ± 0.18	25.79 ± 1.49
250 µg/ml								
24 hours	5.19 ± 0.27	2.64 ± 0.31	10.65 ± 2.86	4.05 ± 0.20	8.72 ± 0.34*	9.16 ± 3.13	1.68 ± 0.13	19.75 ± 1.17
48 hours	4.92 ± 0.16	2.86 ± 0.76	7.88 ± 0.58	3.92 ± 0.29	9.29 ± 0.59*	8.22 ± 1.63	1.43 ± 0.17	26.30 ± 0.93
500 µg/ml								
24 hours	5.16 ± 0.47	3.21 ± 0.58	8.94 ± 1.39	4.46 ± 0.28	9.07 ± 1.04*	8.61 ± 1.72	1.63 ± 0.28	23.79 ± 1.29
48 hours	6.15 ± 0.36	3.08 ± 0.01	8.99 ± 0.26	5.06 ± 0.16	10.03 ± 0.38*	10.43 ± 1.89	1.83 ± 0.10**	29.94 ± 0.90
700 µg/ml								
24 hours	5.99 ± 0.46	2.58 ± 0.09	8.44 ± 0.42	4.42 ± 0.22	10.41 ± 0.49*	7.54 ± 0.55	1.58 ± 0.20	24.84 ± 2.55
48 hours	9.82 ± 0.51*	5.18 ± 0.19*	14.94 ± 0.54	8.97 ± 0.15*	16.19 ± 0.83*	11.30 ± 4.03	2.53 ± 0.13*	31.29 ± 1.68

SD: standard deviation, *$p<0.001$ compared to the control group, **$p<0.05$ compared to the control group

ovarian cancer [27-30]. Our study was conducted on prostate cancer to examine changes in LNCaP cells caused by high doses of AC in the long term and provided significant results concerning the level of APAF-1, which is located in the intrinsic pathway of apoptosis.

Other proteins that control apoptosis in cells are Bcl-2 and Bcl-XL. In a normal cell, these proteins are dominant and are anti-apoptotic markers [31,32]. However, as a result of mutations in the gene encoding these proteins in cancer cells, they are produced in high amounts [33]. Therefore, reducing the levels of these anti-apoptotic markers is important for cancer treatment. An in vitro study examining the effects of herbals on prostate cancer reported that *Melissa officinalis* plant extract inhibited Bcl-2 expression [34]. In another in vitro study conducted with sage plant extracts on a different type of prostate cancer cell (DU-145), there was a significant decrease in Bcl-2 and a significant increase in Bax and
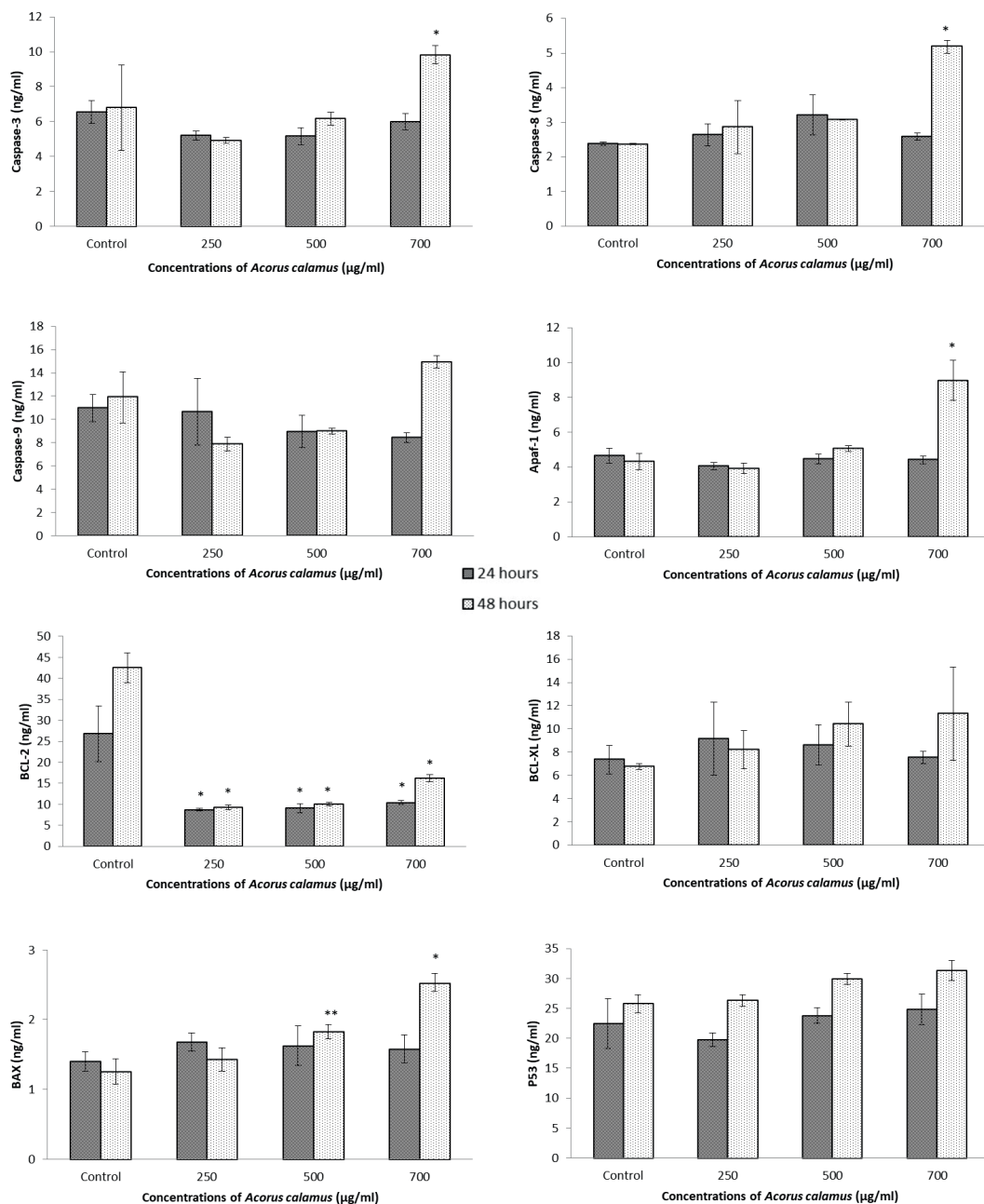
Fig 1. Effects of *Acorus calamus* concentrations on apoptotic markers. *$p<0.001$ compared to the control group, **$p<0.05$ compared to the control group.

caspase-9 [35]. Although the AC plant extract we used in our study did not cause a significant difference between the experimental groups in terms of the Bcl-XL ratio in prostate cancer cells, the significant decrease in Bcl-2 suggests that this plant may be a promising anticancer agent.

Bax, a protein belonging to the Bcl-2 family located in the mitochondrial membrane, is involved in the intrinsic pathway of apoptosis and plays a role in mitochondrial fragmentation [36]. As a pro-apoptotic marker, Bax is one of the important regulators of apoptosis [37]. The Bax/Bcl-2 ratio represents a corresponding change in mitochondrial permeability at the beginning of the induction of apoptosis. Therefore, this ratio is frequently used in cancer research. In clinical and pre-clinical studies, the Bax/Bcl-2 ratio has been investigated in different types of treatments for leukemia, prostate, colorectal, and breast cancers, and it has been suggested that this ratio can be used as a predictive marker in the follow-up of apoptosis potential and response to treatment [38-43]. It has also been used to show the presence of apoptosis in studies on different types of plant extracts and prostate cancer cells. One of these studies reported that the Bax/Bcl-2 ratio increased in prostate cancer cells (PC3) to which *Scutellaria altissima*, which is widely used in China, was applied, and this was accompanied by an increase in sensitivity to cisplatin, an anti-cancer treatment agent [44]. In a study

investigating the effects of a Mediterranean herb, rosemary (*Rosmarinus officinalis*), on prostate cancer cells (PC3), it was found that this plant caused an increase in the ratio of Bax to Bcl-2. [45]. Chen et al. examined the effects of dioscin, an herbal saponin, on LNcaP cancer cells and observed down-regulation of the expression of Bcl-2 and up-regulation of the expression of Bax, reporting similar results to our study in relation to the ratio of these two apoptotic proteins [46]. In our study, Bcl-2 and Bax significantly differed between the experimental and control groups, both when examined alone and in combination as a ratio, which is evidence that AC is also effective in the mitochondrial step of apoptosis in prostate cancer cells.

P53, also under the name of "guardian of the genome", is a transcription factor that is frequently mutated in malignancies [47]. It matters in the regulation of important cellular activities, such as apoptosis, cell cycle, and senescence. It can provide information on the prognosis of cancer [48]. It has also been proven to be one of the major prognostic factors in prostate cancer [49]. Previous publications have proven the presence of p53 mutations in various prostate cancer cells (PC-3, LNCaP, DU-145, and TsuPr-1) [50,51]. Studies using herbal antioxidant compounds, such as resveratrol and berberine, in prostate cancer cells have shown that these herbal compounds increase p53 and thus induce
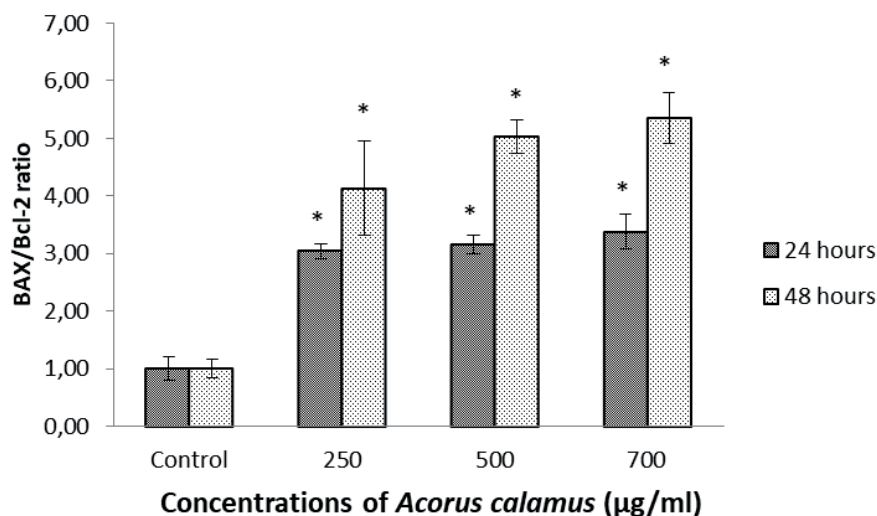
Fig 2. Effects of *Acorus calamus* concentrations on the Bax/Bcl-2 expression ratio. *$p<0.001$ compared to the control group.

apoptosis. In our study, although the p53 levels of the AC-administered groups were found to increase compared to the control group, not significantly different, which may be related to the duration of AC application being limited to 48 hours based on the literature. We consider that a statistically significant result could be achieved in the presence of a longer application time.

One of the most important components of the AC plant that plays a role in the apoptotic process is β-asarone. In a study in which in vivo and in vitro experiments were carried out together, it was found that β-asarone stimulated apoptosis and cell cycle arrest in G1 phase in glioma cells and increased the caspase-3 level [52]. In their in vitro study, Wu et al. found that β-asarone significantly activated caspase-3, -8, and -9 and Bax and suppressed Bcl-2 activity in gastric cancer cells [53]. In a study on colon cancer cells, the authors reported that β-asarone activated the steps of caspase-9 and caspase-3 and also induced apoptosis by decreasing theBcl-2/Bax ratio [54]. Wang et al. showed that β-asarone had effects on apoptotic processes, including caspase-3, caspase-9 activation, Bax up-regulation, and Bcl-2 down-regulation in lung cancer cells, as well as different molecular mechanisms, such as the inhibition of the Wnt/β-catenin signaling pathway [55]. In light of all these literature data and our findings, it is considered that the β-asarone component of AC may be effective in prostate cancer cells.

It has been proven that AC has antioxidant properties due to the presence of asarone in its structure. In a study conducted in rats administered cisplatin, the antioxidant effects of AC on the ovaries were examined, and it was found that AC caused a significant increase in superoxide dismutase levels [56]. AC has also been shown to have antimicrobial and anthelmintic properties [57].

In contrast to the information given above, in a study focusing on cognitive functions, Geng et al. found that β-asarone inhibited the activation of Bcl-2 and caspase-3 in the hippocampus of rats and therefore might be a potential treatment option for dysfunction related to Alzheimer's disease [58]. Similarly, Liu et al. reported that

β-asarone suppressed β-amyloid-induced neuronal apoptosis in the hippocampus of rats [59]. These different results can be attributed to study designs, the in vivo nature of experiments, dosing differences, and these assessments not being undertaken in cancer cells. While apoptosis is insufficient in cancer, excessive apoptosis is seen in neurodegenerative diseases [60]. The results of these studies also suggest that AC acts through different tissue-specific mechanisms and may act as a modulator.

AC has been used in traditional medicine for a long time and is generally considered safe. However, it should be noted that toxic effects can occur with high doses or long-term use. There are various indications in the literature that AC can cause hepatotoxicity, genotoxicity and cardiotoxicity in high doses. Alpha- and beta-asarone have been shown to cause cytotoxicity in human HepG2 liver cells [61]. Asarone has been reported to cause DNA damage and chromosomal abnormalities in vitro [62]. In another study, alpha-asarone was reported to cause cardiotoxicity by inducing cardiac defects and QT prolongation via mitochondrial apoptosis in zebrafish [63]. Most of these toxic effects have been observed at high doses or with long-term exposure. Therefore, caution should be exercised when using the plant and care should be taken not to exceed therapeutic doses. However, further research is needed to standardize, optimize doses and determine the long-term safety profile of AC.

In studies investigating the effect of AC on other cancers, the apoptosis-inducing effect of the alpha-asarone content of AC on lung cancer cells was found [64]. A review paper highlighted that in vitro studies have shown that AC inhibits the growth and proliferation of different cancers such as breast, prostate, colon, lung, liver and leukemia cancers and that these effects are due to the various bioactive components (α-asarone, β-asarone, calamusin, etc.) it contains. In addition, AC has been reported to be associated with mechanisms such as cell cycle arrest, induction of apoptosis and prevention of cell migration [65]. Although in vitro and in vivo studies on the potential role of AC in cancer treatment provide promising evidence, they

need to be substantiated by comprehensive scientific studies before the clinical application of AC can be addressed.

AC appears to have promising anti-cancer effects against prostate cancer and various types of cancer. However, the plant still needs to be supported by extensive scientific research before it can be put into clinical practice.

The studies investigating the effects of AC on normal cell lines have been referenced with the literature. In the study investigating the cytotoxic effect of AC on dermal fibroblast cells from experimental animals, AC did not significantly affect the viability of fibroblast cells even at high concentrations (up to 200 μg/mL) [66]. In the study investigating the effect of AC on human peripheral blood mononuclear cells in vivo, it was found that it did not significantly affect cell viability [67]. These results suggest that the plant may have a selective toxicity potential against cancer cells.

This study did not investigate the potential effects of AC on the tumor microenvironment, particularly MMP-9-mediated remodeling of the extracellular matrix. In future studies, investigating the effects of Achorus calamus on tumor-stroma interactions may help us to better understand the potential of this plant in the treatment of prostate cancer. Another limitation is herbal extracts usually contain several components and there may be synergistic, additive or antagonistic interactions between these components. A phytochemical analysis for AC could not be performed in the study. The composition of herbal extracts may vary depending on factors such as geographical origin, time of harvest and processing methods. A phytochemical analysis could have helped to confirm the composition of the extract and ensure comparability between different studies.

Conclusion

Cancer is a complex and multifactorial disease with a high mortality rate that constitutes a serious threat to human health. In this study, it was observed that AC had an effect on caspases and pro-apoptotic and anti-apoptotic proteins and played a role in both the intrinsic and extrinsic pathways of apoptosis. Significant results observed in the Bax/Bcl-2 expression ratio suggested that it also affected the mitochondrial step of apoptosis. These results were time- and dose-dependent. Our results were compared to those of many studies conducted with different types of herbal medicine on different types of prostate cancer cells. Although the β-asarone component of AC has been shown to have effects on different cancer cells, there are still limited data concerning its effects on prostate cancer. There is a need for further pre-clinical and clinical studies to fully elucidate the molecular mechanism of AC in different types of prostate cancer cells and demonstrate their utility alone or in combination with chemotherapeutic agents used in cancer.

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

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