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ORIGINAL RESEARCH

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Alisma orientale extract inhibits cell proliferation by promoting oxidative stress and apoptosis in prostate cancer cells

Junwei Xu[1], Ning Qin[2,*], Wenbo Jiang[3], Tao Chen[1]

[1]Department of Traditional Chinese Medicine, Affiliated Hospital of Nantong University, 226001 Nantong, Jiangsu, China
[2]Department of Medical Nursing Teaching and Research, Nantong Health College of Jiangsu Province, 226001 Nantong, Jiangsu, China
[3]Department of Cardiovascular Medicine, Suqian Hospital of TCM, 223800 Suqian, Jiangsu, China

***Correspondence**
qinning1635@163.com
(Ning Qin)

Abstract

Prostate cancer is a prevalent malignancy in men, necessitating the development of more effective treatment drugs. Alisma is commonly used in clinical settings for various ailments, including tumors. However, its mechanism of action in prostate cancer cells remains unclear. This study aimed to clarify the effects of Alisma orientale extract (AOE) on prostate cancer. Our results showed that AOE inhibited prostate cancer cell growth and induced apoptosis while promoting oxidative stress. Mechanistically, AOE modulated the p38/nuclear factor kappa-B (NF-κB) pathway to inhibit prostate cancer progression. Taken together, these findings suggest that AOE could serve as a potential treatment for prostate cancer.

Keywords

Prostate cancer; Alisma orientale extract (AOE); Apoptosis; Oxidative stress; p38/NF-κB pathway

1. Introduction

Prostate cancer is a type of common malignancy in men [1]. There are an average of 190,000 new prostate cancer cases worldwide each year, as well as about 80,000 deaths [1, 2]. It is a highly heterogeneous and complex cancer with high mortality and morbidity [2]. Given the indolent nature of prostate cancer in many cases and late-stage diagnosis, improvement in its diagnosis and the development of individualized treatment strategies is essential to improve patient outcomes [3]. In the past few years, there has been an increasing focus on traditional Chinese medicine and plant-based therapies due to their several advantages, such as their mild adverse effects, prolonged therapeutic effects and the ability to target multiple aspects of the disease through small molecule interventions [4]. However, despite these promising aspects, the quest for more effective treatment modalities for prostate cancer patients continues, underscoring the need for ongoing drug development efforts.

Rhizoma alismatis, a plant native to East Asia [5], has been used as a traditional medicine in China [5–7] and is known to play a vital role in numerous medicinal formulations [8]. Clinically, Alisma is used to treat conditions such as diuresis, edema, kidney disease, hyperlipidemia, diabetes, inflammation and tumors [9]. Recent studies have highlighted its notable pharmacological activity against cancer [9]. For instance, Alisma orientale extract (AOE) has demonstrated efficacy in inducing apoptosis in AGS gastric cancer cells [10]. It has also shown promise in inhibiting TNF-α-induced motility expression in MDA-MB-231 breast cancer cells by targeting the inhibitor of kappaB kinase (IKK)-NF-κB-dependent Chemokine C-X-C-Motif Receptor 3 (CXCR3) and Chemokine C-X-C-Motif Ligand 1 (CXCL1) pathways [11]. Moreover, Alisma has been found to enhance the antitumor effects of Bufalin in liver cancer through modulation of the Wnt/β-Catenin axis [11]. Additionally, Alisol B, a triterpenoid derived from Alisma, inhibits melanin production in mouse melanoma cells [12]. Alisol B 23-acetate has been shown to induce autophagy-dependent apoptosis in colon cancer cells through the production of reactive oxygen species (ROS) and activation of the c-Jun N-terminal kinase (JNK) pathway [13]. However, the mechanism of Alisma in prostate cancer cells remains unclear.

This study aimed to elucidate the impact of AOE on prostate cancer. Our results showed that AOE could induce oxidative stress and apoptosis in prostate cancer cells by activating the p38/NF-κB pathway, supporting its potential utility as a therapeutic agent for the treatment of prostate cancer.

2. Materials and methods

2.1 Cell culture and drug treatment

Two distinct types of human prostate cancer cells, namely PC3 and DU145 cells, were bought from American Type Culture Collection (ATCC), routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gbico, Grand Island, NY, USA), and maintained in a controlled environment at 37 °C in an incubator with 5% carbon dioxide (CO_2).

AOE preparation was performed as previously described

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[13]. Briefly, Alisma was extracted with 30% ethanol, followed by two rounds of ultrasound-assisted extractions at 40 °C for 3 hours each. Then, the resulting AOE was filtered and evaporated into a powder and dissolved in medicinal dimethyl sulfoxide (DMSO).

The prostate cancer cells were then incubated with AOE of concentrations 100, 200 and 400 μg/mL for 24 hours.

2.2 Cell counting kit-8 (CCK-8) assay

PC3 and DU145 cells were seeded into 96-well plates and incubated for 48 hours. Subsequently, the cells were treated with CCK-8 reagent (C0037, Beyotime, Beijing, China) for 4 hours, and the optical density at 450 nm (OD450) was measured using a microplate reader (BD).

2.3 Colony formation assay

PC3 and DU145 cells were plated at a density of 1000 cells per well in 6-well plates and cultured in media containing 10% FBS for 14 days at 37 °C. Then, the cells were fixed with paraformaldehyde-PFA (Sigma, Qingpu District, Shanghai, China) for 20 minutes and stained with 0.1% crystal violet (Sigma, Qingpu District, Shanghai, China) for an additional 20 minutes.

2.4 Cell apoptosis assay

PC3 and DU145 cells were fixed with 70% ethanol at −20 °C for 2 hours. To assess cell apoptosis, the fixed cells were stained with propyl iodide (PI) and Fluorescein isothiocyanate isomer I (FITC) Annexin V at 4 °C, and the levels of apoptosis were determined using a flow cytometer (FACS) Calibur flow cytometer (FACSCalibur, BD Biosciences, Inc., Franklin Lake, NJ, USA).

2.5 Immunoblot assays

For protein extraction, the cells were fully lysed with RIPA lysate, and the extracted proteins were quantitated using the bovine serum albumin (BCA) reagent, separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently transferred onto a poly (vinylidene fluoride) membrane. The transferred proteins were blocked using 5% milk for 1 hour. Subsequently, primary antibodies specific to the following targets were added and incubated overnight at 4 °C: Bax (Abcam, ab32503; 1:1000), Bcl-2 (Abcam, ab182858; 1:1000), Cleaved caspase-3 (Abcam, ab32042; 1:1000), p38 (Abcam, ab170099; 1:1000), p-p38 (T180, Abcam, ab178867; 1:500), NF-κB (Abcam, ab209795; 1:1000), p-NF-κB (S337, Abcam, ab28849; 1:500), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (Abcam, ab8245; 1:3000). Lastly, they were incubated with secondary antibodies for 1 hour, and their chemiluminescent signal was captured by photography.

2.6 Reactive oxygen species (ROS), Superoxide dismutase (SOD), glutathione, r-glutamyl cysteingl + glycine (GSH) and myeloperoxidase (MPO) detection

Artery tissues were collected for the quantification of ROS, SOD, GSH and MPO levels using commercial kits (E004-1-1, A001-3-1, A006-2 and A044-1-1, respectively Jiancheng Bioengineering Institute, Nanjing, China). The tissue samples were gently mixed and allowed to undergo the specified reactions by covering them. Subsequently, the OD values were promptly measured using a microplate reader.

2.7 Statistical analysis

The data were assessed using the GraphPad v5.0 software (GraphPad Software, San Diego, CA, USA). Student's *t*-test was employed to determine the statistical significance between two groups, while one-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test was used for multiple comparisons. The data are presented as mean ± standard deviation (SD), with $p < 0.05$ considered statistically significant.

3. Results

3.1 AOE inhibits the growth of prostate cancer cells

Firstly, AOE was administered to prostate cancer PC3 and DU145 cell lines at 100, 200 and 400 μg/mL concentrations for 24 hours. CCK-8 assays revealed that AOE treatment suppressed PC3 and DU145 cell growth, as evidenced by reduced OD450 values (Fig. 1A,B; $p < 0.01$). Furthermore, colony formation assays demonstrated a reduction in the number of colonies formed by PC3 and DU145 cells following AOE treatment, indicating a significant decrease in colony numbers (Fig. 1C,D; $p < 0.01$). Collectively, these findings underscore the inhibitory effects of AOE on the growth of prostate cancer cells, suggesting that AOE could block prostate cancer cell proliferation.

3.2 AOE promotes apoptosis of prostate cancer cells

We then assessed the impact of AOE on prostate cancer cell apoptosis. Flow cytometry (FCM) assays demonstrated a significant increase in the percentage of apoptotic cells in both PC3 and DU145 cells following AOE treatment (Fig. 2A,B; $p < 0.01$). Immunoblot assays revealed that AOE treatment led to reduced Bcl-2 expression and increased levels of Bax and cleaved caspase-3 in both PC3 and DU145 cells (Fig. 2C,D; $p < 0.01$). Overall, these findings indicate that AOE promotes apoptosis in prostate cancer cells.

3.3 AOE promotes oxidative stress in prostate cancer cells

Considering the significance of oxidative stress in prostate cancer progression, we examined the levels of ROS, MDA, GSH and SOD after 24 hours of AOE treatment. The results revealed an increase in ROS and MDA levels, along with a

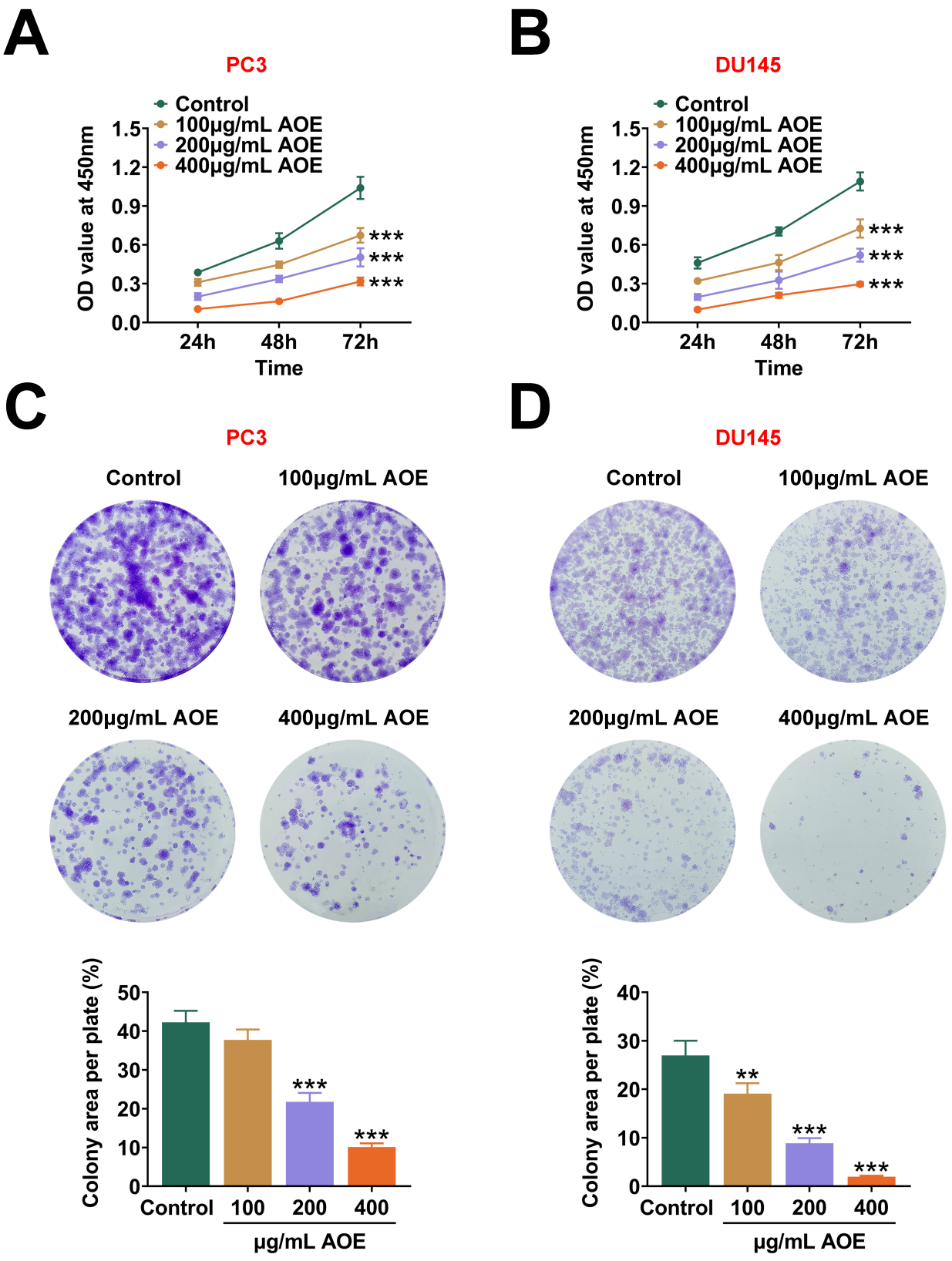

FIGURE 1. AOE inhibits the growth of prostate cancer cells. (A,B) CCK-8 assay results of the proliferation of (A) PC3 and (B) DU145 cells following treatment with 100, 200 and 400 μg/mL AOE, with the corresponding OD450 values shown. (C,D) Colony formation assays showing the colony numbers of (C) PC3 and (D) DU145 cells following treatment with 100, 200 and 400 μg/mL AOE. Data are presented as mean ± SD, $^{**}p < 0.01$, $^{***}p < 0.001$. OD: optical density; AOE: Alisma orientale extract.

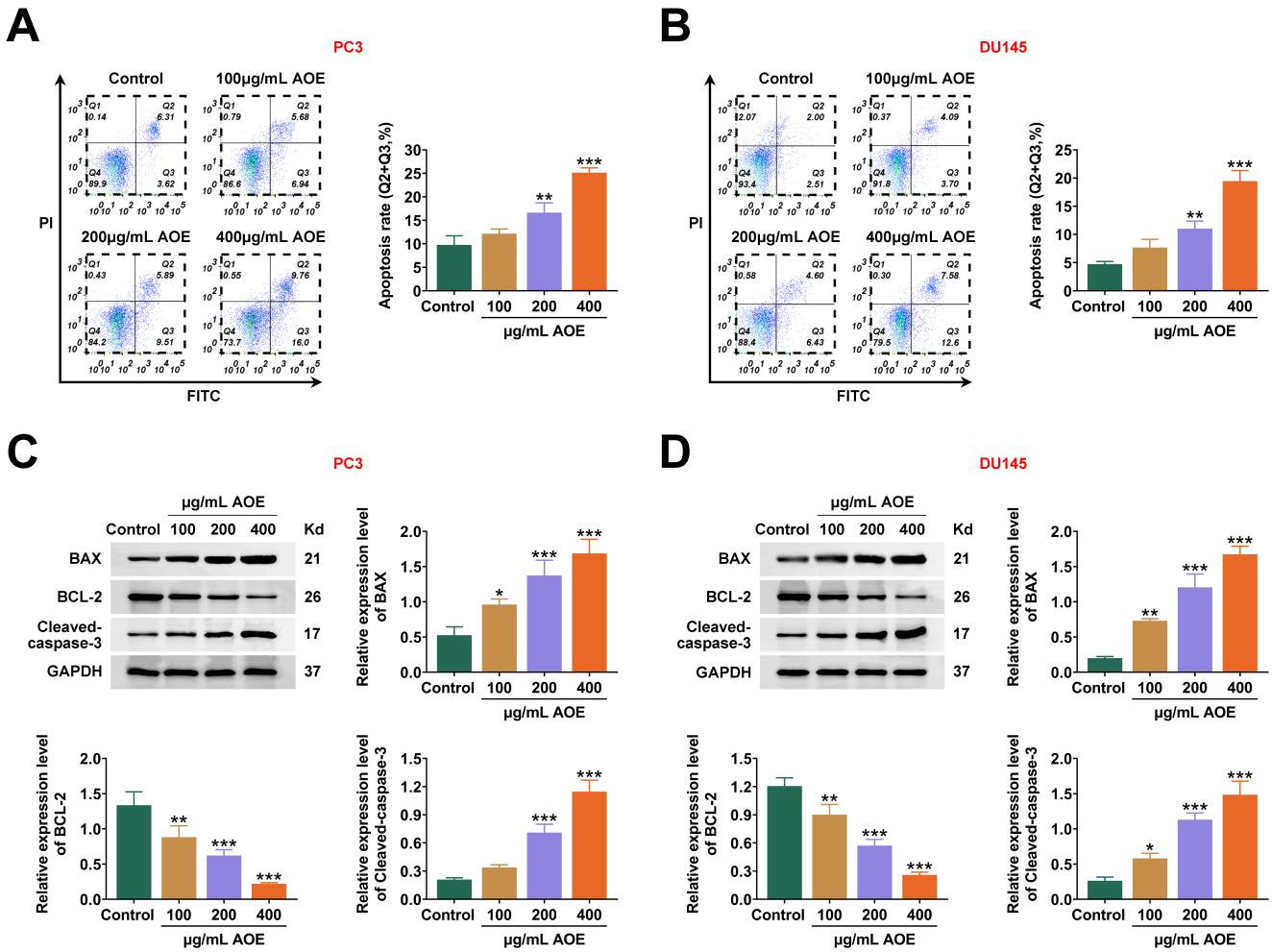

FIGURE 2. AOE promotes apoptosis of prostate cancer cells. (A,B) FCM assay results of the apoptotic degree of (A) PC3 and (B) DU145 cells following treatment with 100, 200 and 400 μg/mL AOE, with the corresponding percentage of apoptosis cells shown. (C,D) Immunoblot assay results and quantification of the expression of cleaved caspase-3, Bax and Bcl-2 in (C) PC3 and (D) DU145 cells treated with 100, 200 and 400 μg/mL AOE. The quantification was shown. Data are presented as mean $\pm$ SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. AOE: Alisma orientale extract; PI: propyl iodide; FITC: Fluorescein isothiocyanate isomer I; GADPH: Glyceraldehyde 3-phosphate dehydrogenase; BCL-2: B-cell lymphoma-2.

decrease in SOD and GSH levels in both PC3 and DU145 cells (Fig. 3A,B; $p < 0.01$), thereby indicating that AOE promotes oxidative stress in prostate cancer cells.

3.4 AOE regulates the p38/NF-κB axis in prostate cancer cells

Lastly, we investigated the mechanism by which AOE suppresses prostate cancer progression. Immunoblot assays revealed that AOE treatment reduced p38 phosphorylation in PC3 and DU145 cells (Fig. 4). Additionally, AOE treatment decreased NF-κB phosphorylation in both cell lines (Fig. 4, $p < 0.01$), confirming its regulatory role in the p38/NF-κB pathway in prostate cancer cells. Thus, it can be deduced that AOE can modulate the p38/NF-κB axis in these cells.

4. Discussion

Prostate cancer is a prevalent urological malignancy, ranking sixth in China and among the top two in Europe and the United States [2]. It is one of the most frequently diagnosed cancers in men [2]. Currently, the conventional treatment approaches for prostate cancer encompass surgery, radiotherapy and chemotherapy, among others, but these modalities have inherent limitations [2]. As a result, researchers have begun to explore targeted therapies for prostate cancer [14]. Drug-based treatment for prostate cancer predominantly involves endocrine drugs [14]. Among these, antiandrogen drugs such as bicalutamide, nilumide and flutamide are commonly used and function by impeding the action of endogenous androgens on prostate cells, thereby delaying the growth or inducing apoptosis in prostate cancer cells [15]. Chinese medicine, guided by a comprehensive holistic perspective, considers the patient's overall well-being, extending beyond the sole focus on cancer. These interventions aim to correct bodily imbalances, eliminate factors contributing to tumor recurrence, and reduce the risk of metastasis. Additionally, Chinese medicine generally exerts minimal harm on healthy cells, avoiding further damage to physical strength during treatment. As the

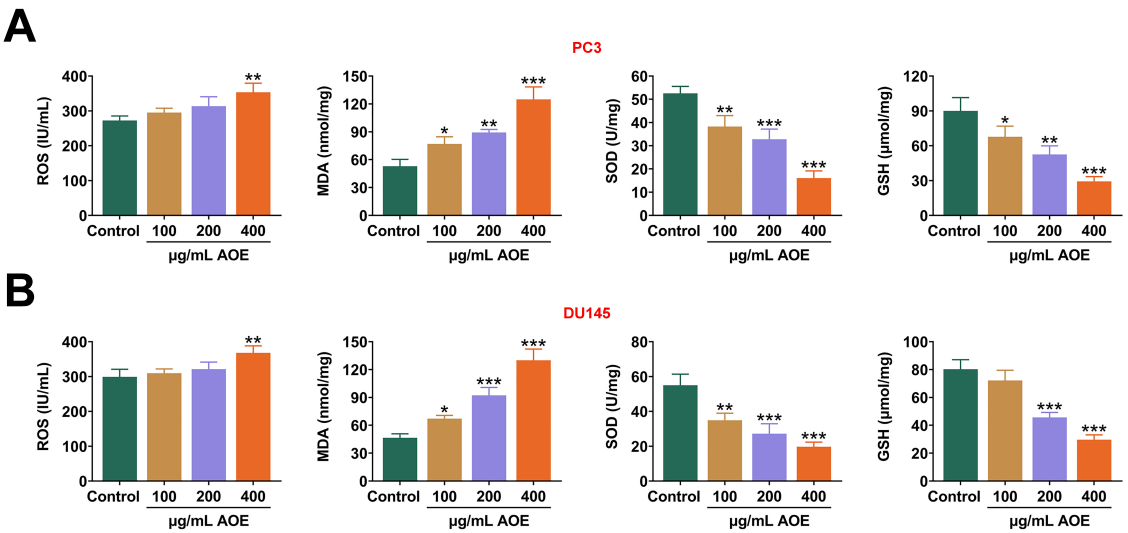

FIGURE 3. AOE promotes oxidative stress in prostate cancer cells. (A,B) ROS levels, MDA, SOD and GSH levels in (A) PC3 and (B) DU145 cells treated with 100, 200 and 400 μg/mL AOE. Data are presented as mean ± SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ROS: reactive oxygen species; AOE: Alisma orientale extract; SOD: Superoxide dismutase; MDA: Malondialdehyde; GSH: glutathione, r-glutamyl cysteingl + glycine.

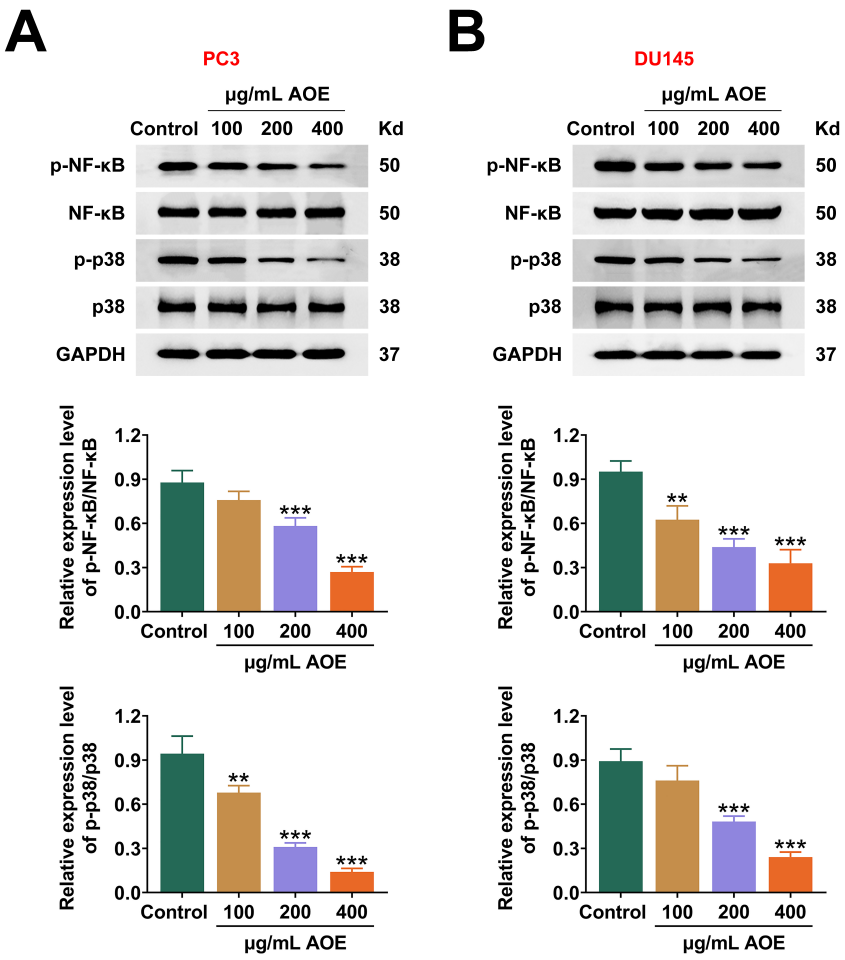

FIGURE 4. AOE regulates the p38/NF-κB axis in prostate cancer cells. (A,B) Immunoblot assay results and quantification of the expression and phosphorylation levels of p38 and NF-κB in (A) PC3 and (B) DU45 cells treated with 100, 200 and 400 μg/mL AOE. Data are presented as mean ± SD, **$p < 0.01$, ***$p < 0.001$. AOE: Alisma orientale extract; NF-κB: nuclear factor kappa-B; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

cancer is targeted by the drugs, the patient's physical strength gradually recovers, and immunity improves [2, 14]. Studies have shown that alisma and its derivatives possess antitumor properties and the ability to affect the behavior of prostate cancer cells. Notably, AOE can hinder cell growth by inducing oxidative stress and apoptosis in these cells, highlighting its potential as a promising candidate for prostate cancer therapy.

AOE, isolated from *rhizoma alismatis*, possesses a range of beneficial effects, such as reducing blood lipids, blood pressure and blood sugar, and also has preventive properties against atherosclerosis and fatty liver [13, 16]. Alisma contains triterpenoids, which can significantly lower cholesterol and triglyceride levels in the blood and aid in averting atherosclerosis and reducing the risk of cardiovascular and cerebrovascular diseases [8, 9]. In recent years, studies have also confirmed that it possesses antitumor effects [11, 12]. It has been demonstrated that a key component of Alisma, Alisol B, exhibits anticancer activity against various cancer cells due to the cytotoxicity and anti-metastatic effects of its alisol compounds [13, 17]. Some of the beneficial functions of triterpenoids present in Alisol B have been shown to be associated with the activation of the p38 pathway and the inhibition of the Phosphatidylinositide 3-kinases (PI3K)/Protein Kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway [13, 17]. In this present study, AOE was found to inhibit cell growth, induce apoptosis and modulate oxidative stress, suggesting its potential as a therapeutic agent for prostate cancer.

In this study, we investigated the impact of Alisma on oxidative stress in prostate cancer cells. Notably, antiandrogen therapy, which deprives prostate cancer cells of androgens, is known to prompt these cells to generate an oxidative stress response [18]. ROS are aerobic metabolites in the body, and various factors, including ionic radiation and anticancer drugs, can induce cell apoptosis through ROS generation [18], aligning with our study's findings. Existing literature indicates that this oxidative stress response is linked to the repeated activation of the androgen receptor within prostate cancer cells, leading to resistance to androgen therapy [18].

Our findings indicate that AOE promotes oxidative stress and apoptosis in prostate cancer cells through the regulation of the p38/NF-κB pathway. This pathway is known to play multifaceted roles, encompassing the regulation of cell survival, differentiation, growth, proliferation, metabolism and motility [19, 20], and mutations in the pathway's components have been associated with the progression of prostate cancer, thereby its activation is closely linked to patient prognosis [19]. Therefore, our study underscores the potential significance of this pathway as a therapeutic target for prostate cancer. However, it is also important to note that this study had some limitations, including the absence of animal-level investigations and in-depth mechanistic studies. Future research could address these limitations to further elucidate the potential of AOE as a treatment for prostate cancer.

5. Conclusions

In summary, AOE can induce oxidative stress and apoptosis in prostate cancer cells by modulating the p38/NF-κB pathway.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

JWX, NQ, WBJ and TC—designed the study and carried them out; supervised the data collection, analyzed the data, interpreted the data; prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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