Biological Activity and Cell Cycle Effects of Natural Extract Kaempferol on Androgen-dependent Prostate Cancer Cells

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Abstract

Background: There is an abnormal enhancement of androgen receptor activity in prostate cancer cells, leading to increased sensitivity to androgens and the promotion of cancer cell proliferation.
Objectives: This study aimed to investigate the effects of *Eucommia ulmoides* leaf-derived kaempferol extract on the biological activity and cell cycle of androgen-dependent prostate cancer cells (LNCaP) through *in vitro* cell culture experiments.
Materials and Methods: Kaempferol was isolated and extracted from the hydrolysate of *E. ulmoides* leaves, and its clearance rates for superoxide anion (O_2^-) and hydroxyl radical (·OH) were analyzed. Subsequently, LNCaP cells were treated with kaempferol at concentrations of 0.0, 0.5, 1.0, and 2.0 μmol L^{-1} and grouped accordingly. Changes in cell proliferation inhibition rate, apoptosis rate, cell cycle distribution, and levels of apoptosis-related proteins were assessed.
Results: The recovery rate of kaempferol from *E. ulmoides* leaves was approximately 98.23%, with kaempferol contents of 0.008% before hydrolysis and 0.089% after hydrolysis. As the concentration of kaempferol increased, the clearing rate (CR) of O_2^- and ·OH free radicals also gradually increased ($p < .05$). In contrast to the 0.0 μmol L^{-1} group, the 0.5, 1.0, and 2.0 μmol L^{-1} groups have exhibited greatly enhanced proliferation inhibition rate and apoptosis rate of LNCaP, a remarkable decrease in the S-phase proportion, a substantial increase in the G2/M-phase proportion, and a significant reduction in Bcl-2 protein. Conversely, expressions of Bax, caspase-3, and caspase-9 were significantly upregulated ($p < .05$).
Conclusion: *E. ulmoides* leaf-derived kaempferol extract demonstrated a pronounced antioxidant effect. Kaempferol was found to inhibit LNCaP proliferation and promote its apoptosis by modulating the cell cycle and the apoptosis-related proteins.

Keywords

Androgen-dependent, apoptosis, cell cycle, kaempferol, prostate cancer

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Introduction

Recently, a consistent increase is evident in prostate cancer (PC) (Cicero et al., 2019). Typically, androgens have the capacity to promote cell growth and proliferation by binding to androgen receptors within the body (Linder et al., 2022). In PC cells, there is an abnormal enhancement of androgen receptor activity, leading to increased sensitivity to androgens and the promotion of cancer cell proliferation (Shiota et al., 2022; Sena et al., 2022). Targeting the androgen signaling pathway is a critical therapeutic strategy for managing androgen-dependent PC.

Eucommia ulmoides, also known as *gutta-percha*, is a plant belonging to the Eucommiaceae family and the genus *Eucommia*. It is a widely used traditional Chinese medicine, and the leaves of *E. ulmoides* possess a broad spectrum of pharmacological properties (Zhao et al., 2022). Previous research has identified five flavonoid compounds isolated from *E. ulmoides* leaves, namely, kaempferol, quercetin, astragalin, hirsutin, and rutin (Hussain et al., 2020). Natural flavonoids have been shown to have anti-inflammatory, antioxidant, and anti-cancer effects in several types of malignant tumors (Iancu et al., 2023; Luo et al., 2023; Tataranu et al., 2023). Kaempferol is an active ingredient in *E. ulmoides* leaves. It possesses antioxidant properties, helping to neutralize free radicals, reduce oxidative stress, and protect the body

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from oxidative damage (Meng et al., 2019). Its antioxidant action contributes to maintaining normal cellular functions and has the potential for anti-inflammatory and anti-tumor effects. Inflammation is a fundamental factor inducing many diseases, including cancer (Neamtu et al., 2022). Overactivation of the inflammatory response can lead to cell damage and abnormal proliferation (Khandia & Munjal, 2020). A study found that kaempferol can inhibit inflammatory pathways and reduce the release of inflammatory cytokines, thus alleviating inflammatory response (Bian et al., 2020). Furthermore, kaempferol exhibits notable anti-tumor activity, inhibiting tumor cell proliferation and invasion while inducing apoptosis (Imran et al., 2019). Qattan et al. (2022) found that kaempferol regulates the cell cycle and modulates the expression of apoptosis-related proteins (ARPs) such as Bcl-2, Bax, and caspase-3 to exert its anti-tumor effects. Wu et al. (2022) demonstrated that kaempferol reverses the resistance of colorectal cancer cells to 5-fluorouracil, potentially by regulating the miR-326-hnRNPA1/A2/PTBP1-PKM2 axis. Ruan et al. (2023) reported that kaempferol impedes the malignant biological behaviors of endometrial cancer, including tumor formation, scar healing, migration, and invasion. It suggests that kaempferol has excellent anti-tumor activity, but its influence on LNCaP proliferation, apoptosis, and cell cycle remains to be determined.

This work involved extracting kaempferol from *E. ulmoides* leaves and evaluating its *in vitro* antioxidant activity. LNCaP was selected as the experimental model to analyze the impacts of kaempferol with varying concentrations on cell proliferation, apoptosis, cell cycle, and expression of ARPs. This work yielded a basis for understanding the mechanisms of kaempferol on androgen-dependent PC.

Materials and methods

Extraction of Kaempferol from E. ulmoides Leaves

20 mg of kaempferol reference standard (SK8030, purity ≥ 98%, Beijing Solarbio Technology Co., Ltd., China) was dissolved in a brown volumetric flask containing anhydrous methanol to prepare a reference standard stock solution at 0.1 mg mL^{-1}. Subsequently, it was diluted to a series of concentrations at 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 16.0, and 20.0 μg mL^{-1} and saved at 4°C. 0.5 g of *E. ulmoides* leaves (Maozhou City Huozhengtang Pharmaceutical Co. Ltd., China) was placed in a round-bottom flask, and a 1:100 sample-to-solvent ratio was achieved by adding anhydrous ethanol–hydrochloric acid (4:1) mixture. The blending solution was hydrolyzed at 70°C for 4 h and adjusted to 50 mL. Following filtration employing a 0.45 μm membrane, content analysis was implemented using an Agilent 1260 infinity II high-performance liquid chromatography (HPLC; Agilent, USA) with a Diamonsil C_{18} column (4.6 × 250 mM^2, 5 μM). A mobile phase with a 0.2% phosphoric acid–methanol (36:64) mixture, with 1.0 mL min^{-1}. After the introduction of 20 μL of the sample, it was detected at 370 nm with the column temperature maintained at 30°C.

Determination of Free Radical Clearing Rate (CR) in Kaempferol

According to the instructions of the free radical scavenging reagent kit (LM80023TK, Shanghai LMAI Biotechnology Co., Ltd., China), 3 mL of Tris–HCl buffer (pH = 8.2) and 0.1 mL of extraction solution with concentrations of 0.0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg mL^{-1} were taken. The mixed solution was equilibrated in a water bath for 20 min. Subsequently, the addition of 0.3 mL of 7 mmol L^{-1} pyrogallol was performed to allow a reaction for 5 min. Afterward, 1.0 mL of 10 mol L^{-1} HCl was introduced to terminate this reaction. The absorbance at 420 nm was assigned to calculate the O_2^- CR. Subsequently, 0.15 mmol L^{-1} $FeSO_4$, 2.0 mmol L^{-1} sodium salicylate, extraction solution, and 6.0 mmol L^{-1} H_2O_2 were sequentially added to generate ·OH through a reaction. The reaction proceeded at 37°C for 1 h. The absorbance value at 510 nm was measured to calculate the ·OH CR.

Culture of LNCaP Cells

LNCaP cells (ORC0499, Shanghai Aoruisai Biological Cell Bank, China) were subjected to a culture in RPMI 1640 culture medium (11875093, Thermo Fisher Scientific, USA) containing 10% heat-inactivated fetal bovine serum (26010074, Thermo Fisher Scientific, USA) and 1% penicillin–streptomycin, incubated in a DHP-9160-ST saturated humidity incubator (Wuxi Marit Technology Co., Ltd., China) at 37°C and containing 5% CO_2 for 48 h before the medium was changed. Afterward, the medium was changed, and the cells were digested using 0.25% trypsin for subculturing. The cells were grouped into four: The control (Ctrl) group, low-concentration kaempferol (LCK) group, medium-concentration kaempferol (MCK) group, and high-concentration kaempferol (HCK) group. LNCaP cells were harvested, digested, and seeded for an additional 24 hours of culture. Cells in Ctrl, LCK, MCK, and HCK groups were cultured in a complete medium consisting of 0.0, 0.5, 1.0, and 2.0 μmol L^{-1} of kaempferol extract from *E. ulmoides* leaves, respectively. Each group had three replicate wells.

Examination of LNCaP Proliferation Inhibition Rate (PIR)

According to the instructions of the 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT) cell proliferation detection kit (11465007001, Sigma–Aldrich, USA), LNCaP cells were grouped and subjected to different treatments. Subsequently, they were cultured under standard conditions for varying durations of 6, 12, 24, 36, and 48 h. Following these incubation periods, 10 μL of a 5 g L^{-1} MTT reagent was introduced. The cells were then incubated again under the standard

culture conditions for an additional 6 h. After discarding the original culture medium, 100 μL of dimethyl sulfoxide (DMSO) reagent was placed and agitated for 10 min. HM-SY96S enzyme-linked immunosorbent assay (Shenzhen Antongda Equipment Technology Co., Ltd., China) was employed to detect the absorbance of each well at a wavelength of 490 nm, and PIR was calculated: PIR (%) = (1 − absorbance in sample group/absorbance in Ctrl group) × 100%.

Detection of Apoptosis Rate (AR) and Cell Cycle of Cells

LNCaP cells were grouped and processed. After routine culture for 24 h, they were digested with trypsin and subjected to a 10-min centrifugation at 1,000 rpm to discharge the supernatant, rinsed three times for 10 min with 0.1 mol L^{-1} pH 7.4 phosphate buffer, and subsequently divided into two portions. One portion was fixed by overnight incubation in prechilled 80% ethanol at 4°C. After washing off the ethanol with phosphate buffer, 10 μL of RNAse and 10 μL of propyridine iodide (PI, ST512, Shanghai Beyotime Biotechnology Co., Ltd., China) were mixed. Subsequently, LNCaP cells were incubated at 4°C without light for 30 minutes. Cell cycle alterations were assessed utilizing a CytoFLEX flow cytometer (Beckman Kurt, USA), with a coefficient of variation correction below 3%. According to the instructions of the Annexin V-FITC cell apoptosis detection kit (C1062M, Shanghai Beyotime Biotechnology Co., Ltd., China), the remaining portion of the cells was resuspended in Annexin V-FITC binding solution. Annexin V-FITC and PI Staining Solution were introduced, thoroughly mixed, and the cells were incubated without light for 15 min. Cell apoptosis was subsequently determined using flow cytometry.

Western Blotting

After routine culture for 24 h, the LNCaP cells were digested with trypsin and subjected to a 10-min centrifugation at 1,000 rpm for discharging its supernatant. Afterward, they were performed with three 10-min washes with 0.1 mol L^{-1} pH 7.4 phosphate buffer and lysed using Radio Immunoprecipitation Assay lysis buffer (R0010, Beijing Solarbio Technology Co. Ltd., China) for an extract of total cellular proteins. Protein concentration in the extracts was quantified using the bicinchoninic acid (BCA) assay method. Subsequently, the proteins underwent SDS-PAGE gel electrophoresis, followed by their transfer onto a PVDF membrane, which was incubated all night at 4°C with Bcl-2 (1:1,000), Bax (1:1,000), caspase-3 (1:1,000), caspase-9 (1:1,000), and GAPDH (1:1,000) protein primary antibody (ab182858, ab32503, ab32351, ab32539, and ab8245, Abcam, UK). Following primary antibody incubation, the membranes were exposed to HRP and IgG (1:1,000) secondary antibodies (ab6759, Abcam, UK) labeled with horseradish peroxidase for 2 h. Signal hybridization was performed using a chemically enhanced luminescence detection kit (P0018FM, Shanghai Beyotime Biotechnology Co., Ltd., China), and bands were visualized and photographed employing a WD-9413A gel imaging system (Beijing Liuyi, China). The grayscale values of the bands were measured using ImageJ, and levels of the target proteins were calculated.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and statistically analyzed with SPSS 22.0. Group differences were compared using *t*-tests and one-way analysis of variance. $p < .05$ and $p < .01$ suggested a statistically great and extremely great significance, respectively.

Results

Detection of Kaempferol Extract

This work suggested that the original kaempferol content was 0.451 μg mL^{-1}, and the recovered kaempferol content was 0.443 μg mL^{-1}, with a recovery rate of approximately 98.23% and a relative SD of approximately 1.980%. Furthermore, this work compared the HPLC chromatograms of the standard sample and the kaempferol extract from *E. ulmoides* leaves before and after hydrolysis. As demonstrated in Figure 1, the kaempferol content sharply increased after hydrolysis. The kaempferol content in *E. ulmoides* leaves before hydrolysis was approximately 0.008%, while it increased to approximately 0.089% after hydrolysis, representing a tenfold increase in content, indicating a remarkable enhancement in the hydrolysis efficiency.

Antioxidant Effect in vitro of Kaempferol Extract from E. ulmoides Leaves

This work evaluated the impact of varying concentrations of kaempferol extract on the CR of O_2^- and ·OH free radicals. As presented in Figure 2, with the increase in kaempferol concentration, both O_2^- and ·OH CR gradually increased. In contrast to the 0 μg mL^{-1} concentration, the O_2^- and ·OH CR at 0.5, 1.0, 2.0, 4.0, and 8.0 μg mL^{-1} sharply increased, showing considerable differences ($p < .05$).

Impacts of Kaempferol Extract from E. ulmoides Leaves on LNCaP Proliferation

This work examined the impact of kaempferol extract at various concentrations on the inhibition of LNCaP cell proliferation. As demonstrated in Figure 3, prolonged time resulted in an enhancement for PIR of LNCaP cells in all groups. The PIR of LNCaP cells after treatment utilizing 0.5, 1.0, and 2.0 μmol L^{-1} kaempferol exhibited great increases with obvious difference to that in the 0.0 μmol L^{-1} group ($p < .01$). Furthermore, this work disclosed that as the concentration of

kaempferol increased, the PIR of LNCaP cells exhibited an increasing trend and showed highly remarkable differences ($p < .01$).

Impacts of Kaempferol Extract from E. ulmoides Leaves on Apoptosis of LNCaP Cells

The impacts of various concentrations of kaempferol extract on apoptosis in LNCaP cells are illustrated in Figure 4. Relative to the 0.0 μmol L^{-1} group, treatment using 0.5, 1.0, and 2.0 μmol L^{-1} kaempferol resulted in a highly observable increase in AR of LNCaP cells ($p < .01$). Meanwhile, this work indicated that as the concentration of kaempferol increased, the AR of LNCaP cells exhibited an increasing trend with highly obvious differences ($p < .01$).

Influence of Kaempferol Extract from E. ulmoides Leaves on the Cell Cycle of LNCaP

This work examined the effects of kaempferol extract with varying concentrations on the LNCaP cell cycle. As explicated in Figure 5, treatment with 0.0, 0.5, 1.0, and 2.0 μmol L^{-1} kaempferol did not alter the G0/G1 phase of LNCaP cells greatly ($p > .05$). In comparison to the 0.0 μmol L^{-1} group, treatment employing 0.5, 1.0, and 2.0 μmol L^{-1} kaempferol effectively shortened the S phase of LNCaP cells, while extending the G2/M phase ($p < .01$). Furthermore, the results of this study indicated that with increasing kaempferol concentration, the S phase of LNCaP cells demonstrated a decreasing trend, while the G2/M phase exhibited an increasing trend ($p < .01$).

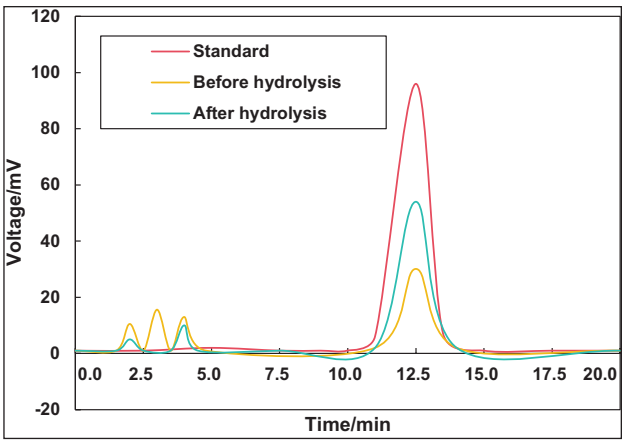

Figure 1. High-performance Liquid Chromatography (HPLC) Spectrogram of Kaempferol Extract from *Eucommia ulmoides* Leaves.

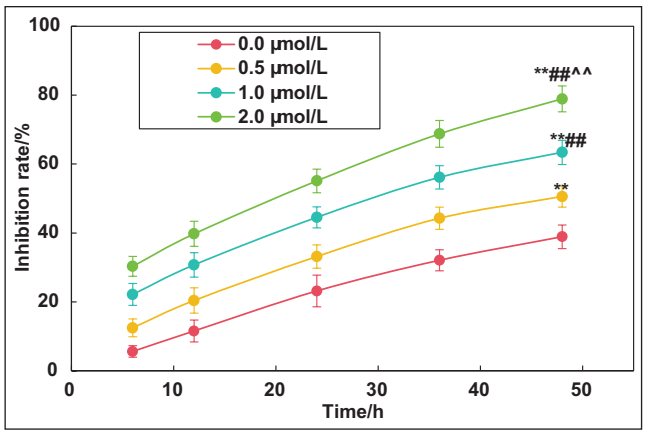

Figure 3. Changes in Proliferation Inhibition Rate (PIR) of LNCaP Cells.

Note: **, ##, and ^^ $p < .01$ compared to the 0.0, 0.5, and 1.0 μmol L^{-1} groups, respectively.

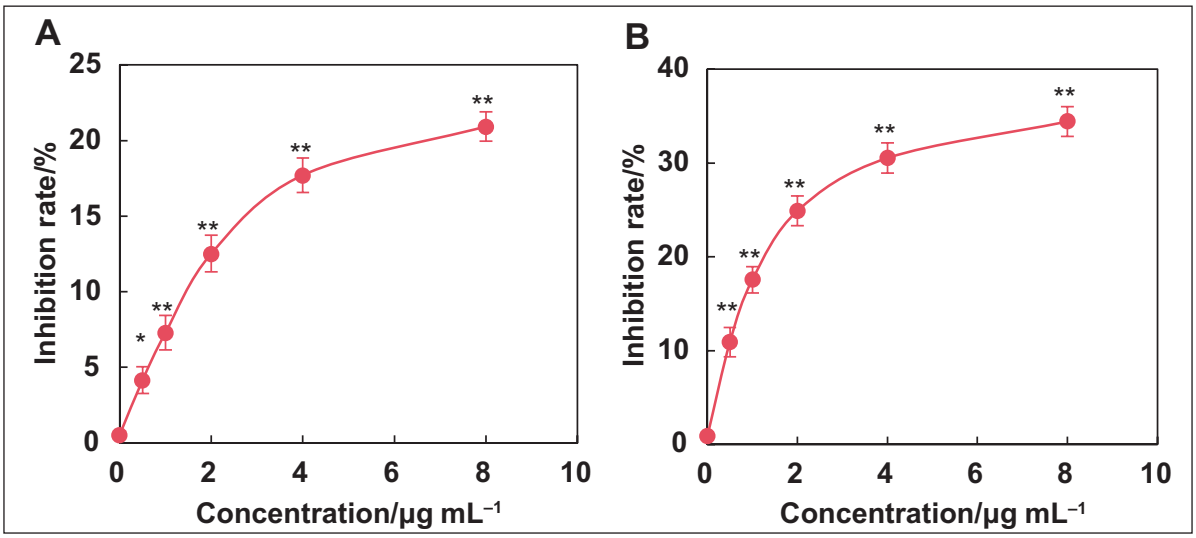

Figure 2. Antioxidant Effect *in vitro* of Kaempferol Extract from *Eucommia ulmoides* Leaves [A: Changes in Clearance Rate (CR) of O_2^- from Distinct Concentrations of Extract; (B): Alternations for CR of ·OH from Varying Concentrations of Extract].

Note: * and ** suggested a substantial difference with $p < .05$ and $p < .01$ in comparison to 0 μg mL^{-1} group, respectively.

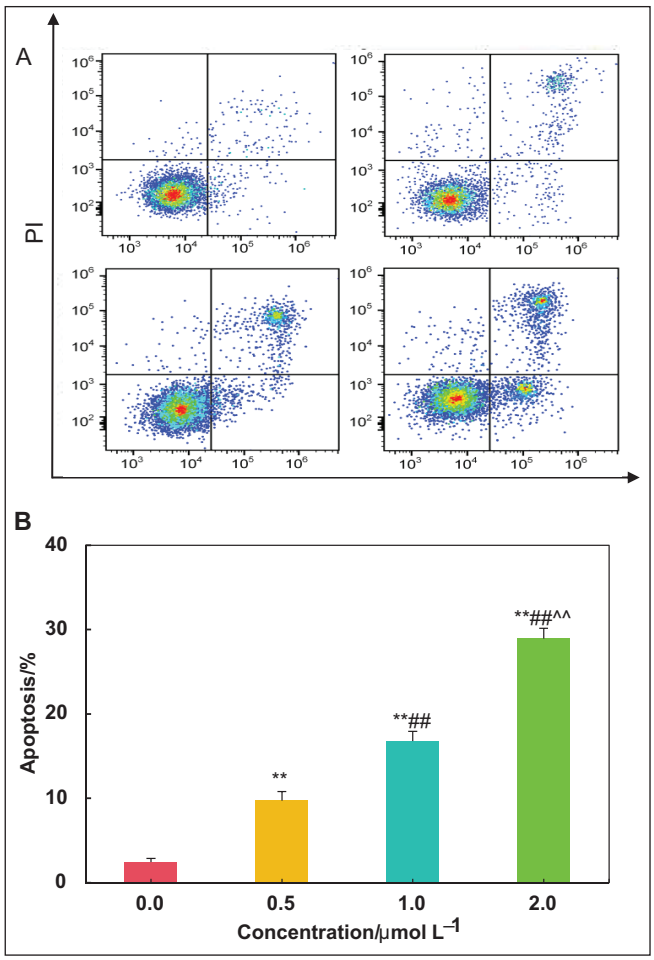

Figure 4. Changes in Apoptosis Rate (AR) of LNCaP Cells (A: FCT Results on Apoptosis; B: AR).

Note: **, ##, and ^^ $p < .01$ compared to the 0.0, 0.5, and 1.0 μmol L^{-1} groups, respectively.

Influence of Kaempferol Extract from E. ulmoides Leaves on LNCaP-related Proteins

This study examined the impacts of kaempferol extract with varying concentrations on levels of ARPs in LNCaP cells, as presented in Figure 6. Treatment using 0.5, 1.0, and 2.0 μmol L^{-1} kaempferol sharply downregulated Bcl-2 protein in LNCaP cells while upregulated Bax, caspase-3, and caspase-9 proteins, demonstrating obvious differences to the 0.0 μmol L^{-1} group ($p < .01$). Additionally, with increasing kaempferol concentration, Bcl-2 protein expression in LNCaP cells decreased, while the Bax, caspase-3, and caspase-9 protein expression experienced an increasing trend ($p < .01$).

Discussion

Previous studies confirmed that after the isolation and purification of *E. ulmoides* leaves extract, the main compounds were quercetin (3,5,7,3′,4′-pentahydroxyflavone), kaempferol (3,5,7,4′-tetrahydroxyflavone), astragalin (kaempferol-*O*-β-D-glucoside), isoquercetin (quercetin-*O*-β-D-glucoside), and rutin (quercetin-3-*O*-rutinoside) (Peng et al., 2021). Flavonoid glycosides can be rapidly absorbed by the human body, and their absorption levels and activity are superior to flavonoid aglycones. In *E. ulmoides* leaves, the major flavonoid glycosides are quercetin and kaempferol (Li et al., 2022; Liu et al., 2023; Wang et al., 2023). In this study, kaempferol was extracted from *E. ulmoides* leaves, and it was observed that the kaempferol content significantly increased after hydrolysis compared to its prehydrolysis state. Therefore, it is suggested that hydrolysis treatment of *E. ulmoides* leaves may yield a more abundant kaempferol extract. Kaempferol exhibits various pharmacological properties including antimicrobial, antioxidant, anti-inflammatory, and anti-tumor effects. Subsequently, this work investigated the antioxidant activity of the kaempferol extract. O_2^- is considered an active oxygen species, and it is the longest-lived free radical in the human body. It can serve as an initiator of free radical chain reactions, leading to the generation of even more potent free radicals (Catani et al., 2023). Superoxide dismutase can eliminate O_2^- free radicals in the body. However, when the activity of superoxide dismutase decreases or when it is produced in excess, O_2^- can cause damage to the body and induce diseases such as atherosclerosis, coronary heart disease, and cancer (Sahoo et al., 2022). ·OH is a highly oxidative oxidant that can cause lipid peroxidation, the breakdown of ribonucleic acids, proteins, and polysaccharides in the body (Sadakiyo et al., 2021). ·OH is also a significant factor in causing DNA damage (Tang et al., 2022). The results of this study demonstrated that the scavenging rates of O_2^- and ·OH radicals increased with the concentration of kaempferol. This suggests that the kaempferol extract from *E. ulmoides* leaves exhibited significant scavenging activity against free radicals and exhibited concentration-dependent behavior.

Under normal circumstances, testosterone promotes the growth and division of cells by binding to intracellular androgen receptors. In PC cells, the abnormal increase in androgen receptor activity leads to enhanced sensitivity to androgens, thereby promoting the abnormal proliferation of PC cells (Hussain et al., 2023; Mekdad et al., 2022). Kaempferol is a plant-derived compound with various pharmacological activities (Yuan et al., 2021). Subsequently, in this study, different concentrations of kaempferol extracts were utilized for the treatment of prostate cancer cells (LNCaP). The results indicated that the proliferation inhibition and ARs of LNCaP cells increased with the concentration of kaempferol. Kaempferol is a natural plant extract, and these results confirm its ability to inhibit the proliferation of LNCaP cells, indicating its characteristics as an anti-PC drug. Inhibition of androgen synthesis or reducing androgen receptor activity is an important strategy for treating androgen-dependent PC (Harris et al., 2022). Apoptosis is crucial for maintaining tissue and organ balance. The apoptosis process in androgen-dependent PC

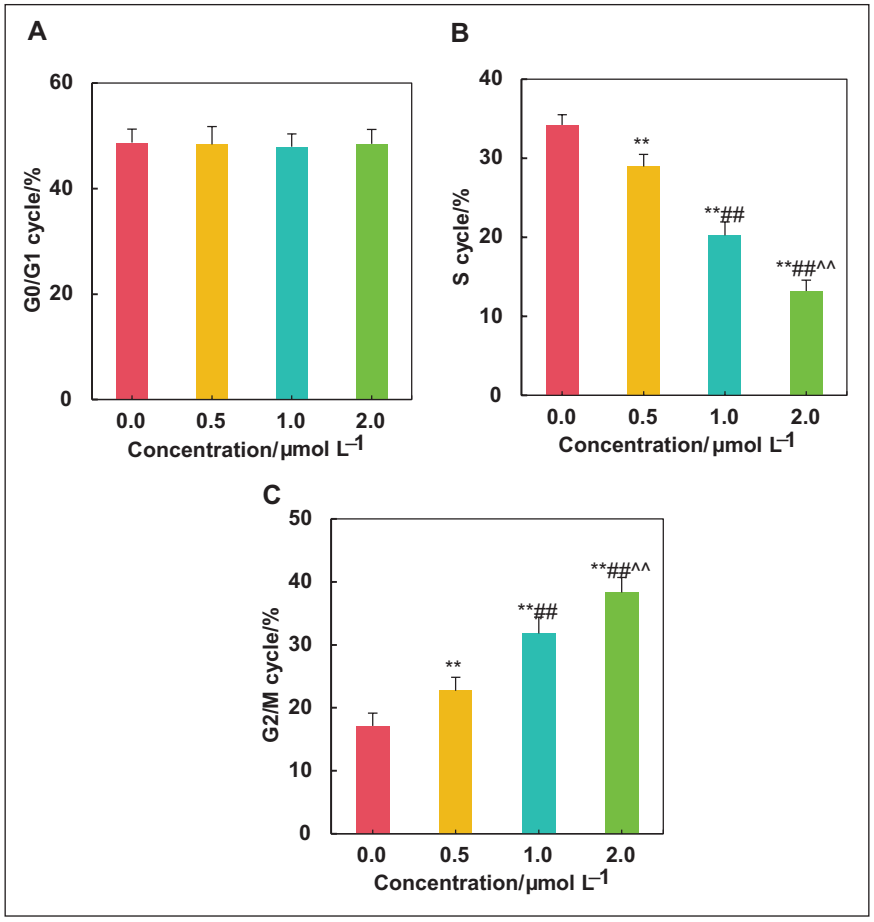

Figure 5. Changes in LNCaP Cell Cycle (A: G0/G1 Phase; B: S Phase; C: G2/M Phase).

Note: **, ##, and ^^ $p < .01$ compared to the 0.0, 0.5, and 1.0 μmol L^{-1} groups, respectively.

cells is disrupted, leading to cancer cells being unable to be eliminated through normal apoptotic pathways, thereby increasing cancer cell proliferation (Wang et al., 2021). Wang et al. (2021) found that kaempferol can mediate Akt/mTOR pathways to apoptosis in pancreatic cancer cells. This suggests that kaempferol may exert its effects on androgen-dependent PC cell growth, proliferation, and apoptosis through various mechanisms, such as inhibiting androgen receptor activity and activating/inhibiting relevant pathways. To better understand the mechanisms by which kaempferol inhibits proliferation and induces apoptosis in LNCaP cells, this work further investigated its effects on the cell cycle of LNCaP cells. The cell cycle is a fundamental process in cellular life activities, and when the cell cycle at a certain phase becomes abnormal, cells may enter related pathological processes. Drugs induce apoptosis by interfering with the cell proliferation cycle, inhibiting normal transitions between cell cycle phases, and promoting apoptosis development (Arora et al., 2023; Patra et al., 2023; Panjwani et al., 2021). The results demonstrated that treatment with kaempferol significantly shortened the S phase of the cell cycle in LNCaP cells while lengthening the G2/M phase. The S phase in the cell cycle is the DNA replication phase, and G2 is the phase before mitosis (Barnaba et al., 2021). This indicates that kaempferol can arrest LNCaP cells in the G2/M phase, disrupt normal transitions between various growth phases of cells, and exhibit a concentration-dependent slowing of cell cycle progression, suppression of cell proliferation, and apoptosis promotion. This aligns with the results of Zhang, Chen, et al. (2022) and Zhang, Qu, et al. (2022), who confirmed that kaempferol can induce androgen-independent PC cells to stall in S and G2 phases and induce apoptosis.

Various genes or proteins regulate the proliferation, migration, invasion, and apoptosis of cancer cells. Bcl-2 and Bax play opposing roles in the regulation of apoptosis in cells. Bcl-2 prevents cell apoptosis by inhibiting apoptosis signal transduction, regulating mitochondrial membrane potential, and protecting mitochondrial function (Ladokhin, 2020). In contrast, Bax promotes cell apoptosis by increasing mitochondrial membrane permeability and releasing apoptotic factors from the mitochondria (Spitz et al., 2022). Caspase-9 is an initiator protein mainly involved in cell apoptosis initiated through the mitochondrial pathway. When apoptotic signals stimulate cells, caspase-9 is activated (Araya et al., 2021). Activated

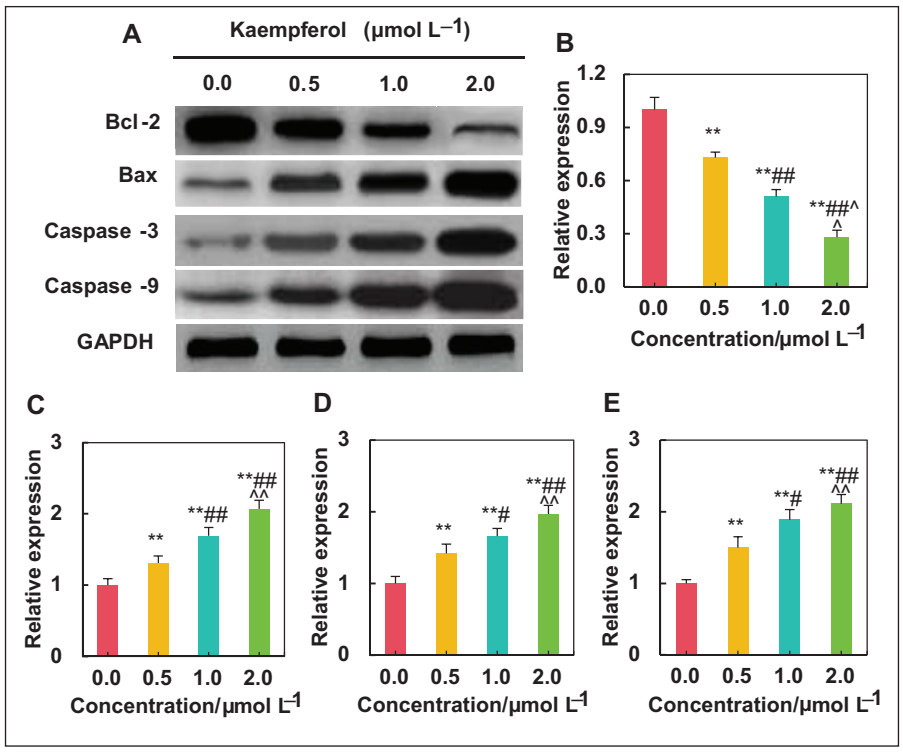

Figure 6. Changes in Apoptosis-related Proteins (ARPs) Levels in LNCaP Cells (A: Western Blotting Results; B: Bcl-2; C: Bax; D: Caspase-3; E: Caspase-9).

Note: **, ##, and ^^ $p < .01$ compared to the 0.0, 0.5, and 1.0 μmol L^{-1} groups, respectively.

caspase-3, as an effector protease, regulates key steps in multiple aspects of cell apoptosis, including DNA damage and fragmentation, remodeling of the cell nucleus and membrane, and degradation of intracellular structures and organelles (Asadi et al., 2022; Stefanowicz-Hajduk et al., 2021; Zhang, Chen, et al., 2022; Zhang, Qu, et al., 2022). The results revealed that following treatment with kaempferol, the expression levels of proapoptotic proteins Bax, caspase-3, and caspase-9 in LNCaP cells increased, while the expression level of anti-apoptotic protein Bcl-2 decreased. This indicates that kaempferol can also regulate proteins related to apoptosis, thereby promoting apoptosis in LNCaP cells.

However, this study also has certain limitations, such as the lack of discussion on the effects of *E. ulmoides* leaf-derived kaempferol extract on the inhibition of PC tumor growth, metastasis, and recurrence *in vivo*. Therefore, future research should involve the establishment of androgen-independent PC animal models to analyze the potential mechanisms of action further following the administration of *E. ulmoides* leaf-derived kaempferol extract.

Conclusion

Kaempferol extract from *E. ulmoides* leaves effectively scavenged O_2^- and ·OH free radicals, demonstrating excellent antioxidant capability. Kaempferol suppressed LNCaP cell proliferation in a concentration-dependent manner and promoted apoptosis primarily through the regulation of ARPs. This work is concerned with analyzing the impacts of kaempferol on the proliferation and apoptosis of LNCaP cells, but further research would be needed to explore its mechanisms of action in treating androgen-independent PC cells. Results in this work offered valuable insights into the therapeutic potential of kaempferol in PC treatment, giving a foundation for developing novel drugs for clinical PC therapy.

Abbreviations

AR: Apoptosis rate; ARPs: Apoptosis-related proteins; BCA: Bicinchoninic acid; PC: Prostate cancer; CR: Clearance rate; Ctrl: Control; DMSO: Dimethyl sulfoxide; HCK: High-concentration kaempferol; HPLC: High-performance liquid chromatography; LCK: Low-concentration kaempferol; MCK: Medium-concentration kaempferol; MTT: 3-(4,5)-Dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide; PIR: Proliferation inhibition rate; TCM: Traditional Chinese medicine; SD: Standard deviation.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

Not applicable.

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