Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Rosemary (*Salvia rosmarinus* Spenn.) and its main components in prostate cancer: A narrative review of anticancer mechanisms

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ARTICLE INFO

Article type:
Review

Article history:
Received: Mar 16, 2026
Accepted: Jul 11, 2026

Keywords:
Apoptosis
Cell proliferation
Inflammation
Oxidative stress
Phytochemicals
Prostatic neoplasms
Rosmarinus
Ursolic Acid

ABSTRACT

Prostate cancer is one of the most aggressive malignancies, marked by late diagnosis, rapid progression, and limited therapeutic options. Resistance to conventional chemotherapy and treatment-related toxicity underscore the need for novel, safe, and effective anticancer strategies. Medicinal plants are increasingly explored as complementary therapies. Rosemary (*Salvia rosmarinus* Spenn.) exhibits antioxidant, anti-inflammatory, and anticancer properties. This narrative review summarizes current evidence on the anticancer effects of rosemary and its main components in prostate cancer, with particular emphasis on underlying molecular mechanisms. A comprehensive literature search was conducted to identify relevant *in vitro*, *in vivo*, and clinical studies published between 2000 and 2026. Electronic databases Scopus, PubMed, and Web of Science were systematically searched to retrieve eligible publications. Rosemary and its main components, including carnosic acid, carnosol, ursolic acid, betulinic acid, and rosmarinic acid, exert multiple anticancer effects in prostate cancer models. They inhibit cell proliferation, induce apoptosis, suppress inflammation and oxidative stress, and interfere with key signaling pathways involved in tumor progression. Moreover, studies report modulation of central oncogenic pathways, including PI3K/Akt/mTOR, NF-κB, STAT3, and AMPK, as well as effects on androgen receptor signaling and cellular metabolism. The findings suggest that rosemary-derived phytochemicals act as multi-target modulators that influence several interconnected processes relevant to prostate cancer development and progression. However, the absence of robust clinical trials remains a major limitation, necessitating future human studies to confirm the safety, pharmacokinetics, and clinical therapeutic value of these promising compounds.

▶ Please cite this article as:
Nakhaei K, Vafadar Moradi E, Sobhani H, Esmaeili S, Rezvani Kakhki B, Jalali J, Ghasemzadeh Rahbardar M. Rosemary (*Salvia rosmarinus* Spenn.) and its main components in prostate cancer: A narrative review of anticancer mechanisms. Iran J Basic Med Sci 2026; 29:

Introduction

Prostate cancer is one of the most common male cancers worldwide, ranking second in incidence after lung cancer. Each year, prostate cancer is responsible for nearly 400,000 male deaths, accounting for approximately 4% of all cancer fatalities in men. The likelihood of developing this disease rises markedly with age, especially among those over 65. Its development has also been associated with diet-related factors, genetic or familial background, obesity, and prior exposure to sexually transmitted diseases. In its early stages, prostate cancer often presents without symptoms, complicating early detection. As the disease progresses, urinary disturbances such as difficulty urinating, increased frequency, back pain, and nocturia may develop. Diagnosis generally involves measuring prostate-specific antigen (PSA) levels in the blood, with values above 4 ng/ml suggesting possible disease and requiring confirmation through prostate tissue biopsy (1).

Prostate cancer is broadly categorized into androgen-sensitive and androgen-insensitive forms. In early disease, tumor growth is driven by androgen receptor signaling, in which androgen binding promotes nuclear androgen receptor activity and the transcription of genes involved in proliferation and PSA production (2), thereby making androgen deprivation therapy effective (3). With disease progression, cancer cells frequently develop resistance to androgen signaling and rely on alternative pathways, most notably the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) axis, which is commonly dysregulated in advanced prostate cancer and promotes survival and proliferation (1). Additional genetic alterations, including mutations affecting tumor suppressors such as phosphatase and tensin homolog (PTEN) and p53, further contribute to

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disease progression (1, 4). Prostate cancer cells also evade apoptosis, highlighting the therapeutic relevance of agents that restore programmed cell death. Treatment strategies vary by disease stage and include surgery, radiotherapy, androgen deprivation, and chemotherapy; while effective, all are associated with significant adverse effects, particularly in advanced or androgen-insensitive disease (1).

Evidence from the literature indicates that a substantial proportion of individuals with cancer turn to complementary or integrative therapies as part of their care. These approaches are often used to alleviate treatment-associated adverse effects, support disease management, and improve symptom control. In response to this trend, modern therapeutic strategies increasingly combine conventional chemotherapeutic regimens with natural compounds and herbal preparations. Such combinations are intended to support physiological homeostasis while potentially suppressing tumor development, progression, and metastatic spread (5).

The herb rosemary (*Salvia rosmarinus* Spenn. Syn. *Rosmarinus officinalis* L.) is commonly used in food processing to enhance flavor and aroma (6). The plant contains several compounds, including caffeic acid, betulinic acid, camphor, carnosic acid, rosmarinic acid, ursolic acid (7), carnosol, and rosmanol (8)(Figure 1).

Physio-pharmacological investigations have revealed that rosemary and main components are associated with a wide range of biological activities, including anti-asthmatic (9), anticancer (10), antidiabetic (11), anti-inflammatory, antioxidant (12), anti-muscle loss (13), anti-obesity (14), cardioprotective (15), hypnotic (16), nephroprotective (17) and neuroprotective (18) effects.

Rosemary is widely used as a natural food preservative and is generally considered safe at customary dietary levels. Nonetheless, consumption should be kept within moderate limits. Reports indicate that high or prolonged intake may be associated with adverse effects on the reproductive system and on renal and hepatic function, and may carry teratogenic risks. In addition, rosemary may interact with certain pharmacological agents, potentially affecting treatment efficacy and safety (10).

This narrative review aims to summarize and critically evaluate the existing evidence on rosemary and its main components in prostate cancer, with particular emphasis on their anticancer mechanisms. Although several review articles have focused on individual rosemary-derived compounds, including carnosic acid, carnosol (19), and ursolic acid (1), no narrative review has comprehensively addressed the whole plant, whose complex phytochemical composition may exert additive or synergistic effects through multi-target modulation of cancer-related pathways. By integrating data from *in vitro* and *in vivo* studies, this review fills an important gap by providing a unified perspective on the mechanistic basis of rosemary's anticancer potential in prostate cancer. In addition, it highlights current limitations, safety considerations, and translational challenges. It underscores the need to bridge the bench-to-bedside gap by developing optimized formulations or nano-formulations and conducting well-designed clinical trials to evaluate rosemary-based interventions as adjunct or complementary therapies to standard prostate cancer treatments.

Methods

Data sources and search procedures

A comprehensive literature search was conducted on January 8th, 2026, to identify studies investigating the anticancer effects of rosemary (S. rosmarinus Spenn.) and its main components in prostate cancer. Three electronic databases, Web of Science, Scopus, and PubMed, were systematically searched for relevant *in vitro*, *in vivo*, and clinical studies published between 2000 and 2026.

The number of records retrieved from each database was as follows: Web of Science (n=250), Scopus (n=332), and PubMed (n=86), giving a total of 668 studies.

The PubMed search query was formulated as follows: ("rosemary"[Title/Abstract] OR "rosmarinus officinalis"[Title/Abstract] OR "salvia rosmarinus"[Title/Abstract] OR "carnosic acid"[Title/Abstract] OR

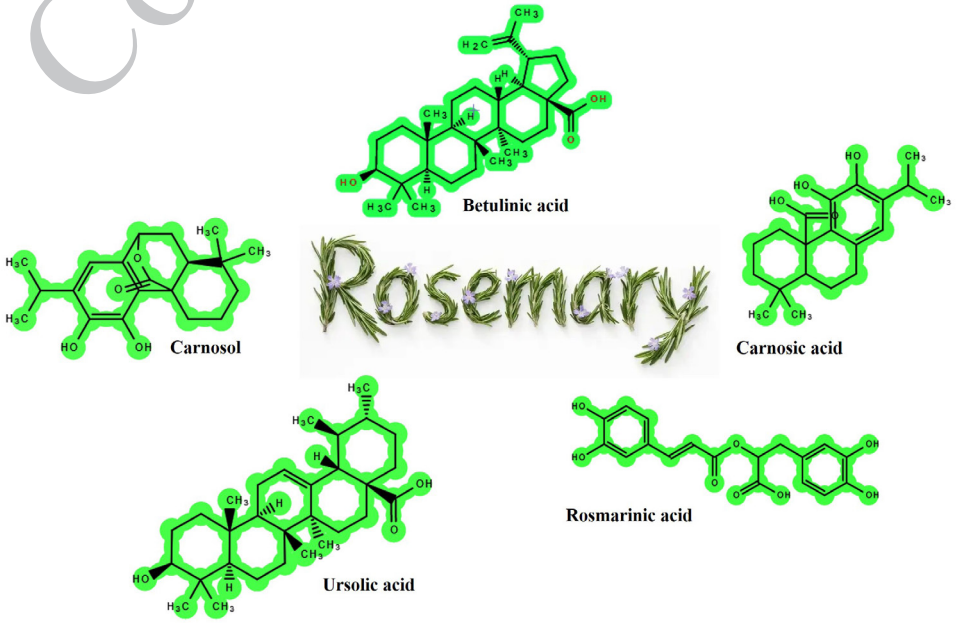

Figure 1. Illustration of rosemary along with the chemical structures of its main components

"carnosol"[Title/Abstract] OR "rosmarinic acid"[Title/Abstract] OR "rosmanol"[Title/Abstract] OR "ursolic acid"[Title/Abstract] OR "betulinic acid"[Title/Abstract]) AND "prostate cancer"[Title/Abstract]

Criteria for study inclusion

Eligible studies were those that aligned with the following predefined conditions:

- Original experimental research involving rosemary or its main components in the field of prostate cancer (including *in vitro*, *in vivo*, or clinical studies).
- Published in English or providing an English abstract.
- Appearing in peer-reviewed journals.
- Presenting sufficient methodological information to permit critical evaluation.
- Reporting outcomes related to cancer prevention, progression, or therapeutic response.

Data refinement and exclusion rationale

Specific exclusion criteria were used to keep the focus on relevant and sound studies. Therefore, the following publications were excluded from the review:

- Conference papers, dissertations, editorials, letters, meta-analyses, review articles, systematic reviews, theses, or other forms of non-peer-reviewed material.
- Research unrelated to prostate cancer or studies that did not examine rosemary or its key chemical constituents.

Study screening and selection process

Following the removal of duplicate records (n=195), 473 unique studies were screened based on titles and abstracts. After this preliminary assessment, 60 articles were retained for full-text evaluation. Upon detailed examination of the complete manuscripts, 46 studies met all inclusion criteria and were included in the final qualitative synthesis (Figure 1).

Current insights into rosemary and its main components in prostate cancer management

Rosemary

In vitro

Evaluating the anticancer effects of rosemary extract across different prostate cancer cell models revealed that treating androgen-insensitive PC-3 human prostate cancer cells with rosemary extract markedly reduced cell survival, suppressed cell proliferation, and inhibited cellular migration. In addition, rosemary extract down-regulated key signaling pathways involved in tumor progression, including the Akt and mTOR signaling pathways. In androgen-sensitive 22RV1 prostate cancer cells, rosemary extract also significantly decreased cellular proliferation and survival. In contrast, no cytotoxic or inhibitory effects were observed in normal prostate epithelial PNT1A cells (20).

A study investigated the cytotoxic effects of aqueous and ethanolic extracts of rosemary leaves on the human prostate cancer cell line DU-145. Cell viability assays demonstrated that both extracts reduced cancer cell survival in a concentration-dependent manner, with the strongest cytotoxic effects observed at 5 mg/ml. The aqueous extract showed greater antiproliferative activity than the ethanolic extract, with a half-maximal inhibitory concentration (IC_{50}) of 1.4140±0.1138 mg/ml. In contrast, the ethanolic extract presented an IC_{50} of 1.8666±0.0367 mg/ml (20).

The collective evidence regarding rosemary extracts

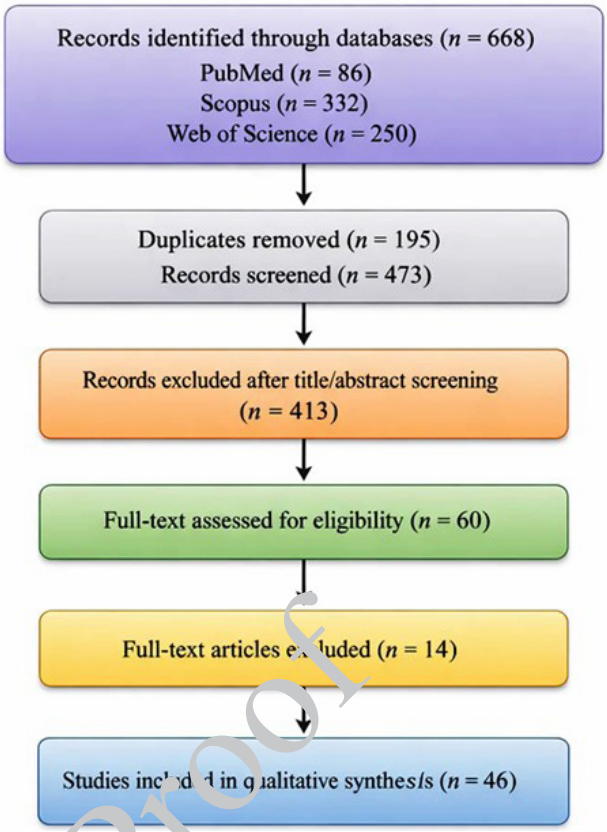

Figure 2. Schematic overview of the literature identification, screening, and selection process for studies evaluating rosemary and its main components in prostate cancer

suggests a multi-faceted protective role against prostate cancer that transcends hormonal status, affecting both androgen-sensitive (22RV1) and androgen-insensitive (PC-3, DU-145) models. A synthesis of current findings indicates that the anticancer activity of these extracts is largely mediated by systemic disruption of the Akt/mTOR signaling axis, a central hub regulating tumor cell survival, proliferation, and metastatic migration (20). Rosemary demonstrates a notable degree of selectivity in its biological activity. Evidence from the reviewed studies indicates that, while it effectively inhibits malignant cell growth, it has minimal effects on normal prostate epithelial cells, including the PNT1A cell line. This pattern suggests a relatively wide therapeutic window, in which rosemary-derived compounds may preferentially target cancer cells while sparing healthy tissue. Such selectivity may be related to metabolic and signaling differences between malignant and normal cells, particularly the greater reliance of cancer cells on anabolic and proliferative pathways.

However, a deeper exploration of the biochemical pathways involved reveals important methodological discrepancies that complicate the translation of these findings into clinical practice. A critical limitation is the high concentrations required to achieve significant cytotoxicity in certain models. For instance, while signaling inhibition is observed in some studies, others report IC_{50} values ranging from 1.4 to 1.8 mg/ml for aqueous and ethanolic extracts (21). From a pharmacological perspective, achieving milligram-per-milliliter concentrations in human plasma through oral supplementation is virtually impossible due

to first-pass metabolism and limited bioavailability. This suggests that the crude extract's *in vitro* potency may not accurately reflect its *in vivo* efficacy unless specific bioactive fractions are isolated or delivery systems are optimized. Furthermore, the observation that aqueous extracts outperform ethanolic ones in certain assays (21) suggests that the most potent anticancer constituents in rosemary may be polar compounds, such as rosmarinic acid, rather than the non-polar terpenoids like carnosic acid that are more prevalent in solvent-based extractions.

Integrating these findings suggests a different perspective on the role of rosemary in cancer therapy. Rather than functioning primarily as a direct cytotoxic agent, rosemary extract may act as a polypharmacological sensitizer. The combined activity of its diverse phenolic and terpenoid constituents may produce a synergistic effect, in which multiple compounds work together to suppress cellular survival pathways that are not fully targeted by single-agent therapies.

Future studies should move beyond high-dose monotherapy approaches and instead explore whether lower, clinically relevant concentrations of rosemary extract can enhance the responsiveness of prostate cancer cells to established treatments. In particular, it would be valuable to examine whether rosemary compounds can sensitize tumor cells to standard-of-care therapies, such as androgen deprivation therapy or other commonly used treatments, thereby improving therapeutic efficacy. Addressing the contradiction between low-dose signaling modulation and high-dose cytotoxicity will be essential; at lower doses, rosemary likely acts as a metabolic regulator, whereas the higher doses reported in the literature trigger nonspecific membrane effects or oxidative stress. Moving forward, clinical translation will require a shift from crude extract studies to standardized, bioavailable formulations that account for the complex synergy between rosemary's polar and non-polar constituents.

Rosemary main components

Betulinic acid

In vitro

To overcome the poor solubility of betulonic acid, a hydrophilic derivative, Boc-glycinated betulonic acid, was chemically synthesized to increase its compatibility with aqueous biological media. Cytotoxicity evaluation revealed that this compound, dissolved in phosphate-buffered saline (PBS) containing ethanol and human serum albumin, markedly suppressed the growth of LNCaP prostate cancer cells, achieving approximately 95.7% inhibition after 72 hr at a concentration of 100 μM, while showing minimal effects on normal fibroblast cells (22).

The therapeutic effect of betulinic acid on a prostate cancer cell line was examined, and the data showed that after 48 hr of exposure, changes in cellular sensitivity were observed, as indicated by cell viability and cell death (23).

Treating prostate cancer cells with betulinic acid led to cellular events commonly associated with apoptosis, including increased reactive oxygen species (ROS) production, oxidative deoxyribonucleic acid (DNA) damage, and DNA fragmentation; however, these effects occurred without the formation of topoisomerase I–DNA cleavable complexes, which are typically involved in ROS-mediated apoptotic pathways. Further analyses demonstrated that betulinic acid directly interacts with cellular topoisomerase I and prevents its binding to oxidatively damaged DNA. *In vitro* experiments using oligonucleotides containing 8-oxoguanosine showed that betulinic acid inhibited topoisomerase I-mediated DNA cleavage. Moreover, suppression of the topoisomerase I gene using small interfering ribonucleic acid (siRNA) reduced staurosporine- and camptothecin-induced cell death but had little effect on betulinic acid-induced cytotoxicity (24).

Experimental evaluation using lymph node carcinoma of the prostate (LNCaP) cells revealed that betulinic acid reduced expression of vascular endothelial growth factor (VEGF) and the anti-apoptotic protein survivin. These anti-angiogenic and pro-apoptotic effects were associated with activation of the proteasome-dependent degradation of the transcription factor specificity protein (Sp)1, Sp3, and Sp4. These transcription factors are known regulators of VEGF and survivin expression and are frequently overexpressed in tumor tissues (25).

It has been reported that exposing PC-3 prostate cancer cells to betulinic acid reduced the DNA-binding activity of nuclear factor-kappa B (NF-κB) and lowered nuclear levels of the NF-κB p65 subunit. The inhibitory effect was associated with diminished activity of inhibitor of κB kinase (IKK) and reduced phosphorylation of inhibitor of κB alpha (IκBα) at serine residues 32 and 36, leading to subsequent degradation of IκBα. Reporter gene analyses further indicated that betulinic acid suppressed NF-κB–dependent transcriptional activity. These molecular changes were accompanied by pro-apoptotic alterations, including an increased ratio of B-cell lymphoma-2–associated X protein (Bax) to B-cell lymphoma-2 (Bcl-2) and cleavage of poly(adenosine diphosphate-ribose) polymerase (PARP). In addition, betulinic acid blocked tumor necrosis factor alpha (TNF-α)–induced activation of NF-κB through the IκBα signaling pathway, thereby enhancing the sensitivity of the cancer cells to TNF-α–mediated apoptosis (26).

The findings of another study on PC-3 cells demonstrated that betulinic acid suppressed the expression and transcriptional activity of hypoxia-inducible factor-1 alpha (HIF-1α) under hypoxic conditions. In parallel, betulinic acid inhibited hypoxia-stimulated activation of signal transducer and activator of transcription 3 (STAT3), as evidenced by reduced phosphorylation, diminished DNA-binding capacity, and decreased nuclear accumulation of this transcription factor. A marked reduction in both intracellular and secreted levels of VEGF, a key angiogenic mediator and downstream target gene of STAT3, accompanied these inhibitory effects. Functional assays further showed that conditioned medium from hypoxic PC-3 cells treated with betulinic acid impaired capillary-like tube formation in human umbilical vein endothelial cells (HUVECs), indicating anti-angiogenic potential. Further analysis confirmed that betulinic acid disrupted the binding of HIF-1α and STAT3 to the VEGF promoter region. Moreover, silencing STAT3 using siRNA further potentiated the betulinic acid-mediated suppression of VEGF production during hypoxia (27).

Assessing the anticancer activity of betulinic acid on the ubiquitin–proteasome system in prostate cancer indicated that betulinic acid suppressed the activity of several deubiquitinases, enzymes responsible for removing ubiquitin from proteins, leading to the accumulation of

polyubiquitinated proteins and subsequent reduction of multiple oncogenic proteins. This disruption of protein homeostasis was associated with enhanced apoptotic cell death in prostate cancer cells. In contrast, normal fibroblasts did not exhibit inhibition of deubiquitinase activity or accumulation of polyubiquitinated proteins following betulinic acid treatment, which corresponded with the absence of cytotoxic effects in these normal cells (28).

Experiments in androgen-responsive LNCaP cells and androgen-independent DU145 cells showed that betulinic acid treatment reduced cell survival and increased the ratio of Bax to Bcl-2. This alteration promoted mitochondrial cytochrome c release, caspase activation, and PARP cleavage, ultimately leading to apoptotic cell death. In LNCaP cells, betulinic acid also enhanced the stabilization of the tumor suppressor protein p53 through increased phosphorylation at serine 15, while no such effect was observed in DU145 cells. Additionally, the expression of the cyclin-dependent kinase inhibitor p21/Waf1 was elevated in both cell types. Betulinic acid treatment further suppressed the NF-κB signaling pathway by reducing the phosphorylation of inhibitor of kappa B kinase alpha (IKKα) and IκBα, which limited the nuclear translocation and DNA-binding activity of the NF-κB p65 subunit and promoted its accumulation in the cytoplasm (29).

Another study was designed to assess the anticancer activity of betulinic acid, its cytotoxic effects, and its ability to induce endoplasmic reticulum stress in prostate cancer cell lines derived from African American and Caucasian patients. Treatment with betulinic acid and its ionic derivatives significantly reduced the viability of all tested prostate cancer cells, and the observed cytotoxic activity was unaffected by either the cells' ethnic origin or their androgen responsiveness. Although betulinic acid compounds affected proteins associated with endoplasmic reticulum stress, these effects were relatively limited when compared with classical endoplasmic reticulum stress inducers such as tunicamycin and thapsigargin. Furthermore, the expression of glucose-regulated protein 78 (GRP78) showed a strong association with the levels of CCAAT/enhancer-binding protein homologous protein (CHOP) and cleaved PARP/caspase-3 in prostate cancer cells (30).

Research on betulinic acid explored approaches to enhance its anticancer efficacy against prostate cancer by combining it with ceranib-2 and incorporating the drugs into a nanoscale delivery system. In this approach, a nanocarrier composed of a zinc-doped manganese dioxide (Zn:MnO_2) nanocomposite core coated with a gallic acid–polylactic acid (PLA)–alginate polymeric shell was developed to improve the delivery of the hydrophobic compound. *In vitro* cytotoxicity assessment using PC-3 human prostate cancer cells demonstrated that free betulinic acid and ceranib-2 individually showed negligible antiproliferative activity. In contrast, nano-encapsulated formulations significantly enhanced the anticancer effect, where betulinic acid/Zn:MnO_2@ gallic acid-PLA-alginate nanocarriers, betulinic acid–ceranib-2/Zn:MnO_2@ gallic acid-PLA-alginate nanocarriers, and the free betulinic acid–ceranib-2 combination exhibited IC_{50} values of 6.498, 7.351, and 18.571 µg/ml, respectively (31).

A study investigating derivatives of betulinic acid developed twelve ureidic derivatives through a one-pot chemical modification process. These synthesized compounds were evaluated for their anticancer potential against PC-3 cells. Among the tested molecules, the derivative designated BA1 exhibited the strongest cytotoxic activity, with an IC50 of 3.87 µM against PC-3 cells, compared with the reference chemotherapeutic agent doxorubicin. Further findings indicated that BA1 suppressed cellular proliferation and metastatic behavior while promoting apoptosis in a prostate cancer cell line (32).

A nanoemulsion formulation containing betulinic acid and *Calotropis procera* seed oil was designed to investigate its potential for anticancer applications. The cytotoxic activity of the formulation was assessed *in vitro* by exposing PC3 and mouse fibroblast cells (L929). The results indicated selective cytotoxicity toward cancer cells, with IC_{50} values ranging from 4.6 to 6.2 µg/ml in PC3 cells, while substantially higher IC_{50} values (>75 µg/ml) were observed in fibroblasts (33).

In vivo

Assessing the effect of Boc-lysinated betulonic acid *in vivo* in male athymic mice bearing human LNCaP xenograft tumors demonstrated that intraperitoneal administration of Boc-lysinated betulinic acid resulted in about a 92% reduction in tumor growth compared with untreated controls. Histological examination indicated that tumor suppression was associated with mitotic arrest and induction of apoptosis, as verified by detection of cleaved caspase-3 in ex vivo tumor cultures (22).

In vivo evidence from the transgenic adenocarcinoma of the mouse prostate (TRAMP) model illustrated that administration of betulinic acid significantly suppressed the growth of primary prostate tumors and was associated with increased apoptosis, as well as decreased angiogenesis and cellular proliferation. Additionally, betulinic acid treatment reduced the expression of androgen receptor and cyclin D1 in tumor tissues. Betulinic acid inhibited deubiquitinase activity and promoted the accumulation of ubiquitinated proteins in prostate tumors, whereas normal mouse tissues showed no significant changes in apoptosis or protein ubiquitination (28)(Table 1).

Generally, betulinic acid has demonstrated significant potential as an anticancer agent, particularly in prostate cancer (Figure 3). The studies reviewed highlight its complicated mechanisms of action, predominantly centered on the induction of apoptosis and the inhibition of pathways crucial for tumor growth and survival. A recurring theme in multiple *in vitro* studies is betulinic acid's ability to trigger programmed cell death via various pathways. For instance, it has been shown to increase ROS production, leading to oxidative DNA damage and fragmentation, a cascade that ultimately culminates in apoptosis (24). While this pathway often involves topoisomerase I, betulinic acid appears to exert its effects independently of the formation of topoisomerase I–DNA cleavable complexes, instead directly interacting with topoisomerase I to prevent its binding to oxidatively damaged DNA, suggesting a novel interaction mechanism.

Furthermore, betulinic acid influences key regulatory pathways involved in cancer progression. Its ability to suppress VEGF expression and the anti-apoptotic protein survivin through proteasome-dependent degradation of the transcription factors Sp1, Sp3, and Sp4 underscores its anti-angiogenic and pro-apoptotic potential (25). The NF-κB signaling pathway is another critical target, with betulinic

acid effectively reducing NF-κB DNA-binding activity and nuclear translocation by inhibiting IKK and reducing IκBα phosphorylation. This inhibition not only promotes apoptosis by altering the Bax/Bcl-2 ratio and inducing PARP cleavage but also blocks TNF-α-induced NF-κB activation, thereby sensitizing cancer cells to apoptosis (26, 29). The interference with NF-κB is particularly significant, as this pathway is frequently dysregulated in cancer, promoting inflammation, survival, and metastasis.

Under hypoxic conditions, a common feature of solid tumors, betulinic acid has been observed to suppress the expression and transcriptional activity of HIF-1α and STAT3. This inhibition reduces VEGF levels, indicating a potent anti-angiogenic effect that starves the tumor of nutrients and oxygen (27). The disruption of the ubiquitin-proteasome system, specifically the suppression

Table 1. Biological effects of rosemary extracts and betulinic acid on prostate cancer cells and animal models

Compound	Study design	Doses/Duration	Results	Ref.
Rosemary extracts				
In vitro				
Rosemary extract	Androgen-insensitive PC-3 cells and androgen-sensitive 22RV1 cells	-	↓ Cell survival, proliferation, migration, Akt and mTOR signaling pathway	(18)
Rosemary aqueous and ethanolic extracts	DU-145 cells	0.625-10 to mg/ml	↓ Cancer cell survival	(17)
Betulinic acid				
In vitro				
Betulinic acid	Prostate cancer cell line	0-20 µg/ml	↓ Cell viability and cell death	(20)
Betulinic acid	DU145 cells	0-25 µmol/l	↑ Apoptosis, ROS production, oxidative DNA damage, DNA fragmentation; ↓ Cellular topoisomerase I binding to oxidatively damaged DNA	(21)
Betulinic acid	LNCaP cells	1–20 µmol/l	↑ Activation of proteasome-dependent degradation of Sp1, Sp3, and Sp4 ↓ Expression of VEGF and survivin	(30)
Betulinic acid	PC-3 cells	1–40 µM	↓ Degradation of IκBα, Bax/Bcl-2 ratio, PARP cleavage, TNF-α–mediated apoptosis ↓ DNA-binding activity of NF-κB, nuclear levels of the NF-κB p65 subunit, activity of IKK, phosphorylation of IκBα at serine residues 32 and 36, NF-κB–dependent transcriptional activity	(22)
Betulinic acid	PC-3 cells	0-40 µM	↓ Expression and transcriptional activity of HIF-1α under hypoxic conditions, hypoxia-stimulated activation of STAT3, intracellular and secreted levels of VEGF	(23)
Betulinic acid	DU145, LNCaP, and PC3 cells	2.5, 5, 7.5, and 10 µM	↑ Accumulation of polyubiquitinated proteins, apoptotic cell death ↓ Activity of several deubiquitinases, level of multiple oncogenic proteins	(24)
Betulinic acid	Androgen-responsive LNCaP cells and androgen-independent DU145 cells	10 and 20 µM	↑ Bax/Bcl-2, mitochondrial cytochrome c release, PARP cleavage, activation of caspases, apoptosis, expression of the cyclin-dependent kinase inhibitor p21/Waf1, p53 stabilization ↓ Cell survival, NF-κB signaling pathway, phosphorylation of IKKα, and IκBα, nuclear translocation and DNA-binding activity of the NF-κB p65 subunit	(25)
Betulinic acid and its ionic derivatives	DU145, LNCap, and PC-3 cell lines derived from African American and Caucasian patients	10, 30, 50 µM	↑ Apoptosis, activation of caspase-3, and cleavage of PARP ↓ Viability of all tested prostate cancer cells	(26)
Derivatives of betulinic acid	PC-3 cells	-	BA1: ↑ Cytotoxic activity, apoptosis ↓ Cellular proliferation, metastatic behavior	(28)
In vivo				
Boc-lysinated betulonic acid	Male athymic mice bearing human LNCaP xenograft tumors	30 mg/kg, 17 days, i.p.	↑ Mitotic arrest, apoptosis ↓ Tumor growth	(19)
Betulinic acid	TRAMP transgenic mice	5, 10 mg/kg, 11 times in 14 days, i.p.	↑ Apoptosis, accumulation of ubiquitinated proteins in prostate tumors ↓ Growth of primary prostate tumors, angiogenesis, cellular proliferation, expression of androgen receptor and cyclin D1 in tumor tissues, deubiquitinase activity	(24)

Akt: protein kinase B; Bax: B-cell lymphoma-2–associated X protein; Bcl-2: B-cell lymphoma 2; DNA: deoxyribonucleic acid; HIF-1α: hypoxia-inducible factor-1 alpha; i.p.: intraperitoneal; IKK: inhibitor of κB kinase; IκBα: inhibitor of κB alpha; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor kappa B; PARP: poly(adenosine diphosphate-ribose) polymerase; Ref: reference; ROS: reactive oxygen species; STAT3: signal transducer and activator of transcription 3; TNF-α: tumor necrosis factor-alpha; VEGF: vascular endothelial growth factor

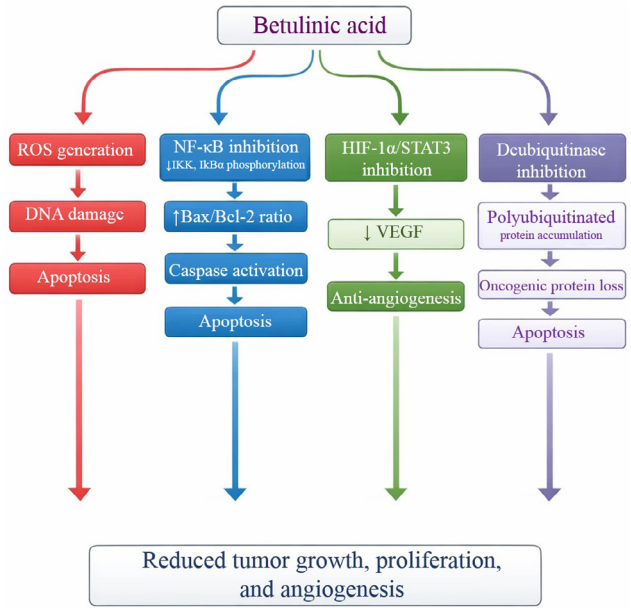

Figure 3. Multi-target anticancer mechanisms of betulinic acid in prostate cancer

Betulinic acid exerts antitumor activity through several interconnected molecular pathways. First, it induces ROS generation, leading to oxidative DNA damage and apoptosis. Second, betulinic acid inhibits the NF-κB signaling pathway by suppressing IKK activity and IκBα phosphorylation, thereby increasing the Bax/Bcl-2 ratio, activating caspases, and promoting apoptotic cell death. Third, it suppresses hypoxia-associated signaling by inhibiting HIF-1α/STAT3 activity, resulting in decreased VEGF expression and reduced angiogenesis. Fourth, betulinic acid disrupts the ubiquitin–proteasome system by inhibiting deubiquitinases, leading to accumulation of polyubiquitinated proteins and loss of oncogenic proteins, ultimately triggering apoptosis. Collectively, these mechanisms converge to inhibit tumor cell proliferation, suppress angiogenesis, and reduce overall tumor growth in prostate cancer models

of deubiquitinase activity, leading to the accumulation of oncogenic proteins and subsequent apoptosis, presents another distinct mechanism of action for betulinic acid (28). This highlights betulinic acid's ability to interfere with fundamental cellular processes that maintain protein homeostasis, which is often exploited by cancer cells.

The *in vivo* studies support these findings, demonstrating significant tumor growth inhibition in xenograft models and transgenic mouse models of prostate cancer. Bocylysinated betulinic acid, a more soluble derivative, achieved substantial tumor reduction accompanied by mitotic arrest and apoptosis (22). Similarly, betulinic acid treatment in the TRAMP model led to decreased tumor growth, angiogenesis, and cellular proliferation, alongside increased apoptosis and reduced expression of androgen receptor and cyclin D1, further supporting its therapeutic efficacy in a more complex biological system (28).

Critically evaluating the methodologies, several studies utilized specific prostate cancer cell lines like LNCaP, PC-3, and DU145, which represent different aspects of prostate cancer, including androgen-responsiveness and independence. However, the tested concentrations varied significantly across studies, ranging from low micromolar to millimolar ranges for derivatives. While some studies employed cell viability assays and apoptosis markers (e.g., cleaved caspase-3, PARP cleavage, Bax/Bcl-2 ratio), the direct measurement of ROS production and DNA damage provides more mechanistic insights. The use of siRNA to suppress specific genes (e.g., STAT3, topoisomerase I) strengthens the causal links between betulinic acid action and observed outcomes.

An important limitation across many *in vitro* studies is the potential lack of direct translatability of high concentrations used to achieve cytotoxicity. For instance, achieving 95.7% inhibition at 100 μM of a derivative (22) raises questions about achievable therapeutic levels *in vivo*. However, the authors did address this by developing a more soluble derivative. Moreover, while betulinic acid has shown minimal effects on normal fibroblast cells in some studies (22, 28), the selective toxicity needs to be strongly established across a wider range of normal cell types. Some studies noted that the effects of betulinic acid were not influenced by ethnic origin or androgen responsiveness (30), suggesting a broad applicability, but further validation in diverse patient populations is necessary.

Contradictions or inconsistencies in findings, such as the observation that betulinic acid did not induce significant endoplasmic reticulum stress compared with classical inducers (30), suggest that although endoplasmic reticulum stress markers may be affected, it may not be the primary or sole mechanism. The findings regarding p53 stabilization being observed in androgen-responsive LNCaP cells but not in androgen-independent DU145 cells (29) point to potential differences in cellular context that could influence therapeutic response.

Despite these promising results, moving betulinic acid from the laboratory to the clinic is challenging, mainly due to its poor aqueous solubility and low bioavailability. Strategies to overcome this, such as developing hydrophilic derivatives (22), nano-delivery systems (31), and nanoemulsion formulations (33), are crucial. These advanced formulations have demonstrated improved effectiveness and greater selectivity, highlighting their potential for future clinical development. The combination of betulinic acid with other agents, such as ceranib-2, also suggests a potential for synergistic therapeutic effects, which could allow for lower doses and reduced toxicity.

Synthesizing these results, a novel perspective could involve exploring the interplay between the various pathways affected by betulinic acid. For example, the suppression of NF-κB and HIF-1α could synergistically reduce VEGF production, thereby not only inhibiting angiogenesis but also potentially mitigating the pro-survival signals mediated by NF-κB within the tumor microenvironment. Furthermore, disruption of the ubiquitin-proteasome system may have downstream effects on the stability of key transcription factors such as Sp1, Sp3, Sp4, and even mutated p53, thereby amplifying apoptotic signals. A significant future direction could be to investigate the impact of betulinic acid on the tumor immune microenvironment, as pathways like NF-κB are critical regulators of immune cell infiltration and function. Understanding whether betulinic acid can modulate immune responses, perhaps by reducing immunosuppressive factors or enhancing anti-tumor immunity, could open up new therapeutic strategies, potentially in combination with immunotherapies. Realizing the full therapeutic promise of betulinic acid in prostate cancer will depend on creating more stable and targeted delivery systems.

Carnosic acid

In vitro

Carnosic acid demonstrated significant anti-proliferative

effects against androgen-independent human prostate cancer PC-3 cells. The inhibitory effect increased with both concentration and exposure time and was primarily attributed to the induction of apoptosis. Mechanistically, apoptotic induction was accompanied by activation of caspase-3, caspase-7, caspase-8, caspase-9, and an elevated Bax/Bcl-2 ratio, mitochondrial release of cytochrome c, and reduced expression of inhibitor of apoptosis (IAP) proteins. In addition, carnosic acid suppressed the IKK/NF-κB signaling pathway, as indicated by reduced DNA-binding activity, decreased nuclear translocation of p50 and p65 subunits, and lowered IκBα phosphorylation. This suppression was linked to inhibition of Akt phosphorylation and kinase activity, together with enhanced activity of protein phosphatase 2A (PP2A). Furthermore, similar pro-apoptotic effects were observed in androgen-refractory prostate cancer DU145 cells through the intrinsic pathway, evidenced by caspase-3 activation, PARP cleavage, increased Bax/Bcl-2 ratio, and cytochrome c release (34).

In human prostate cancer cell lines LNCaP and 22Rv1, carnosic acid treatment reduced androgen receptor protein levels and suppressed the expression of PSA. This effect was accompanied by dose-dependent up-regulation of endoplasmic reticulum stress markers, including binding immunoglobulin protein (BiP) and CHOP. Additional experiments showed that co-treatment with the proteasome inhibitor MG-132 or the endoplasmic reticulum stress modulator salubrinal reversed carnosic acid-induced androgen receptor loss, supporting the involvement of endoplasmic reticulum stress–mediated proteasomal degradation. Moreover, carnosic acid triggered endoplasmic reticulum stress in prostate cancer cells while sparing normal prostate epithelial cells obtained from patient biopsies (35) (Table 2).

A study evaluating the effects of carnosic acid on prostate cancer cell lines DU145 and PC-3 showed that the compound exerted minimal anticancer activity. Treatment with carnosic acid at micromolar concentrations for 48 hr caused only slight reductions in cell viability and no significant apoptotic or necrotic effects. In long-term assays, carnosic acid produced little or no inhibition of clonal growth and induced only a modest decrease in S-phase cells without clear G0/G1 arrest. Mitochondrial measurements revealed a minor decline in membrane potential in DU145 cells and no effect in PC-3 cells, while oxidative stress markers remained unchanged. At the molecular level, serum- and

Table 2. Biological effects of carnosic acid, carnosol, and rosmarinic acid on prostate cancer cells and animal models

Compound	Study design	Doses/Duration	Results	Ref.
		Carnosic acid		
		In vitro		
Carnosic acid	PC-3 and DU145 cells	0-100 μM	↑ Anti-proliferation, apoptosis, activation of caspase-3, caspase-7, caspase-8, caspase-9, Bax/Bcl-2 ratio, mitochondrial release of cytochrome c, Expression of IAP proteins, IKK/NF-κB signaling pathway, nuclear translocation of p50 and p65 subunits, IκBα phosphorylation, Akt phosphorylation	(31)
Carnosic acid	LNCaP and 22Rv1 cells	0-100 μM	↑ Endoplasmic reticulum stress markers ↓ Androgen receptor protein levels, PSA expression	(32)
Carnosic acid	DU145 and PC-3 cells	0-100 μM	↓ Cell viability, S-phase cells, mitochondrial membrane potential	(33)
Carnosic acid	PC-3 and LNCaP cells	2.5-60 μM	↑ Phosphorylation and activation of AMPK, activation of ACC and sestrin-2 ↓ Cell survival and proliferation, phosphorylation and activation of mTOR, Akt	(34)
		Carnosol		
		In vitro		
Carnosol	PC3 cells	10-70 μM	↑ Cell cycle arrest at the G2 phase ↓ Cell survival, PI3K/Akt signaling	(35)
Carnosol	LNCaP and 22Rv1 cells	0-60 μM	↓ Protein expression levels of androgen receptor and estrogen receptor alpha	(36)
Carnosol	LNCaP and DU145 cells	0, 0.5, 4 μmol/l	↑ Caspase-3 enzyme activity ↓ Cell viability, mRNA and protein levels of Sonic Hedgehog and Gli1, nuclear localization of Gli1	(37)
		In vivo		
Carnosol	Athymic (nu/nu) male nude mice with 22Rv1 prostate cancer xenograft	30 mg/kg, 5 times/week, 28 days, p.o.	↓ Tumor growth, PSA levels	(36)
		Rosmarinic acid		
		In vitro		
Rosmarinic acid	PC-3 and DU145 cells	25-300 μM	↑ Early and late apoptosis, P53, cyclin-dependent kinase inhibitor p21, Bax, caspase-3 activation, PARP-1 cleavage ↓ Cell proliferation, colony formation, tumor spheroid development, expression of HDAC2, PCNA, cyclin D1, cyclin E1, Bcl-2 expression	(38)

ACC: acetyl-CoA carboxylase; Akt: protein kinase B; AMPK: adenosine monophosphate-activated protein kinase; Bax: B-cell lymphoma-2–associated X protein; Bcl-2: B-cell lymphoma 2; Gli1: glioma-associated oncogene homolog 1; HDAC2: histone deacetylase 2; IAP: inhibitor of apoptosis; IKK: inhibitor of κB kinase; mRNA: messenger ribonucleic acid; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor-kappa B; p.o.: per os (oral); PARP-1: poly(adenosine diphosphate-ribose) polymerase-1; PCNA: proliferating cell nuclear antigen; PI3K: phosphoinositide 3-kinase; PSA: prostate-specific antigen; Ref: reference

glucocorticoid-regulated kinase-1 (SGK1) expression was unchanged in DU145 but slightly increased in PC-3, though protein levels and phosphorylation at serine-422 (Ser422) modestly declined after treatment (36).

A study showed that carnosic acid dose-dependently reduced cell survival and proliferation in PC-3 and LNCaP cells, with IC50 values of approximately 64 μM in PC-3 cells and 21 μM in LNCaP cells. Mechanistic analysis revealed that carnosic acid suppressed the phosphorylation and activation of mTOR, Akt, and ribosomal protein S6 kinase beta-1 (p70 S6K), indicating inhibition of the Akt–mTOR signaling pathway, which is known to regulate cell growth and survival. In contrast, treatment with carnosic acid enhanced the phosphorylation and activation of adenosine monophosphate-activated protein kinase (AMPK), along with increased activation of acetyl-CoA carboxylase (ACC) and its upstream regulator sestrin-2 (37).

In vivo

In animal xenograft models of prostate cancer, treatment with carnosic acid led to significant degradation of the androgen receptor and increased CHOP expression in tumor tissues. These molecular changes were accompanied by a marked reduction, approximately 53%, in tumor growth compared with untreated controls, thereby corroborating the therapeutic potential of the compound against prostate cancer in a living organism (35).

In summary, carnosic acid does not function merely as a direct cytotoxic agent but rather as a multi-target regulator that weakens prostate cancer cell survival by concurrently interfering with oncogenic signaling networks, metabolic balance, and cellular protein quality control mechanisms. When the available *in vitro* and *in vivo* evidence is synthesized, a logical mechanism emerges in which carnosic acid disrupts critical survival networks, most prominently the Akt signaling axis, while concurrently inducing mitochondrial, endoplasmic reticulum, and metabolic stress within prostate cancer cells. Suppression of Akt signaling appears to play a central role in these effects, as it connects several downstream processes, including inhibition of NF-κB–mediated transcription, reduction of mTOR-dependent anabolic growth, and promotion of mitochondrial apoptosis through increased Bax/Bcl-2 ratios, cytochrome c release, and activation of caspases (34, 37). This convergence on Akt suggests that carnosic acid acts as a network-level disruptor of prostate cancer cell resilience rather than targeting a single pathway.

A second major protective mechanism involves disruption of androgen receptor signaling through induction of endoplasmic reticulum stress and proteasome-dependent androgen receptor degradation. By up-regulating BiP and CHOP and promoting androgen receptor loss in androgen-responsive cells, carnosic acid directly suppresses PSA expression. It attenuates a pathway that remains clinically relevant even in advanced or therapy-resistant disease (35). When considered alongside AMPK activation and mTOR inhibition, these findings suggest a broader stress-adaptation model in which carnosic acid forces prostate cancer cells into an energetically unfavorable state while simultaneously impairing their ability to maintain oncogenic protein homeostasis. A novel implication of this integrated stress response is that carnosic acid may sensitize prostate cancer cells to existing therapies, particularly androgen-deprivation strategies or PI3K/Akt-mTOR inhibitors, by lowering the adaptive threshold that typically underlies therapeutic resistance.

Despite this mechanistic consistency, conflicting reports regarding the anticancer efficacy of carnosic acid point to important methodological limitations. Studies reporting minimal effects generally employed lower micromolar concentrations (≤10 μM)(36), whereas strong antiproliferative and pro-apoptotic effects were observed at higher concentrations, often exceeding 20-70 μM (37). This dose dependency raises concerns regarding pharmacological relevance, as such concentrations may be difficult to achieve systemically in humans without formulation optimization. Variability in experimental endpoints, lack of standardized positive controls, and limited assessment of long-term adaptive responses further complicate cross-study comparisons. Nevertheless, although individual studies have focused on different molecular endpoints, the available evidence collectively implicates several recurring pathways, including Akt/NF-κB, mTOR/AMPK, and androgen receptor signaling, in the biological activity of carnosic acid.

Translationally, the *in vivo* xenograft evidence demonstrating androgen receptor degradation, CHOP induction, and substantial tumor growth inhibition supports therapeutic potential (35). However, these models do not fully capture the complexity of human disease. Taken together, the data suggest that carnosic acid is unlikely to function optimally as a monotherapy but may have greater value as a metabolic and signaling sensitizer within combination regimens. Future studies focusing on bioavailability, clinically achievable dosing, and rational drug combinations could clarify whether the multi-stress–inducing properties of carnosic acid can be applied to improve prostate cancer treatment outcomes.

Carnosol

In vitro

Exposing PC3 cells to carnosol demonstrated that this compound interferes with several signaling pathways associated with tumor development. Cell viability analysis indicated that carnosol reduced the survival of PC3 cells in both dose- and time-dependent manners. Moreover, treatment with carnosol induced cell cycle arrest at the G2 phase. To explore the underlying mechanisms, a protein array analyzing 638 signaling proteins was performed, which identified AMPK as a key target. Activation of this pathway was associated with downstream alterations in the mTOR/heat shock protein 70 S6 kinase (HSP70S6k)/eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) signaling cascade. Moreover, carnosol was found to suppress PI3K/Akt signaling in a dose-dependent manner (38).

In vitro experiments using prostate cancer cell lines LNCaP and 22Rv1 showed that treatment with carnosol reduced the protein expression levels of androgen receptor and estrogen receptor alpha (39).

The effect of carnosol was examined on both hormone-dependent LNCaP and hormone-independent DU145 prostate cancer cell lines, as well as tissue specimens, to determine the expression of pathway markers Sonic Hedgehog and glioma-associated oncogene homolog 1 (Gli1). Elevated levels of Sonic Hedgehog and Gli1 were observed in prostate cancer tissues compared with normal

tissues. When cells were treated with graded concentrations of carnosol, a dose-dependent suppression of cell viability in both LNCaP and DU145 lines was detected. Further analysis confirmed that carnosol reduced messenger ribonucleic acid (mRNA) and protein levels of Sonic Hedgehog and Gli1, with more pronounced inhibition in LNCaP cells than in DU145 cells. The compound also diminished nuclear localization of Gli1, further demonstrating blockade of Hedgehog signaling activity. Additionally, carnosol treatment enhanced caspase-3 enzyme activity in a concentration-dependent manner (40).

In vivo

In vivo evaluation of 22Rv1 prostate cancer xenograft mouse models showed that oral carnosol (30 mg/kg, five times per week for 28 days) significantly inhibited tumor growth by approximately 36% and reduced circulating prostate-specific antigen (PSA) levels by about 26% (39) (Table 2).

Commonly, available evidence indicates that carnosol exerts anticancer activity against prostate cancer through the simultaneous modulation of several oncogenic signaling pathways (Figure 4). Experimental findings collectively show that carnosol reduces prostate cancer cell viability, induces G2-phase cell cycle arrest, and promotes apoptosis, suggesting an overall suppression of tumor cell proliferation (38, 40). These effects appear to involve activation of AMPK, together with inhibition of PI3K/Akt signaling. Downstream suppression of the mTOR pathway and its

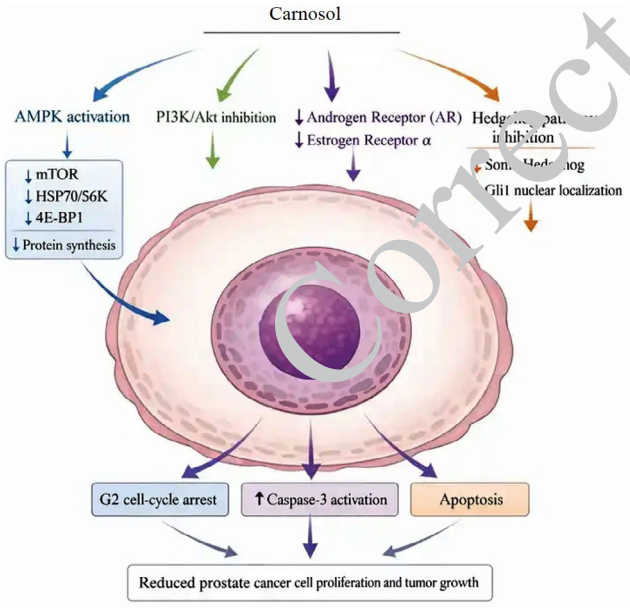

Figure 4. Mechanistic illustration of the anticancer effects of carnosol in prostate cancer cells

Schematic representation of the molecular mechanisms through which carnosol modulates prostate cancer cell signaling. Within the cellular environment, carnosol activates AMPK, which in turn suppresses the mTOR signaling axis and its downstream translational regulators, including S6 kinase and 4E-BP1, thereby limiting protein synthesis and cellular growth. In parallel, carnosol inhibits the PI3K/Akt pathway, thereby reducing survival signaling. Carnosol also down-regulates hormone-related signaling pathways by decreasing androgen receptor (AR) and estrogen receptor-α expression. Furthermore, the compound suppresses the Sonic Hedgehog/Gli1 signaling pathway and reduces nuclear localization of Gli1, indicating inhibition of Hedgehog pathway activity. These coordinated signaling alterations lead to G2-phase cell cycle arrest, activation of caspase-3–mediated apoptosis, and decreased prostate cancer cell viability. Together, these mechanisms contribute to reduced tumor proliferation and growth in prostate cancer models

translational regulators further indicates that carnosol may restrict anabolic growth processes in prostate cancer cells (38). In parallel, modulation of hormone-related signaling, including reduced androgen receptor and estrogen receptor alpha expression, and inhibition of the Sonic Hedgehog/Gli1 axis suggest that carnosol interferes with pathways involved in tumor progression, stemness, and hormone responsiveness (39, 40).

The integration of these findings supports the hypothesis that carnosol may exert its anticancer activity by coordinately disrupting signaling networks that sustain prostate cancer cell survival. A plausible mechanistic framework is that AMPK activation not only suppresses mTOR-dependent protein synthesis but also indirectly attenuates androgen receptor signaling and Hedgehog pathway activity through metabolic and transcriptional reprogramming. Such network-level interference may explain the observed cell cycle arrest and induction of apoptosis across different prostate cancer cell lines. This multi-target profile could confer a therapeutic advantage over single-pathway inhibitors, which often trigger compensatory pathway activation and drug resistance. From a pharmacological perspective, carnosol may therefore function as a metabolic and signaling "re-programmer," shifting cancer cells from a proliferative to a stress-responsive state.

Despite these promising findings, several limitations should be considered. Most evidence derives from *in vitro* studies using relatively high micromolar concentrations, and the pharmacologically achievable levels of carnosol in human tissues remain unclear. In addition, many studies rely primarily on signaling or expression analyses without extensive mechanistic validation through genetic or pathway-specific interventions, which limits definitive conclusions about causal targets. The available *in vivo* evidence is also limited to a small number of xenograft experiments showing moderate tumor growth inhibition and reduced prostate-specific antigen levels following oral administration of carnosol (39). While these results support biological activity in living systems, they do not yet establish long-term efficacy or safety.

The available *in vivo* evidence remains limited but encouraging. Oral administration of carnosol in a xenograft mouse model resulted in moderate tumor growth inhibition and reduced circulating prostate-specific antigen levels (39), supporting the biological relevance of the *in vitro* observations. However, the antitumor effect was partial rather than curative, and the study duration was relatively short. Moreover, the lack of detailed pharmacodynamic analyses in tumor tissues limits the ability to directly link the observed tumor suppression to the molecular mechanisms identified in cell culture models.

From a translational perspective, current evidence suggests that carnosol may be more suitable as an adjuvant or complementary therapeutic agent rather than a stand-alone treatment. Its ability to concurrently modulate key pathways, including AMPK, PI3K/Akt, androgen receptor, and Hedgehog signaling, indicates potential for enhancing the effectiveness of existing treatments such as androgen deprivation therapy or targeted kinase inhibitors by limiting pathway redundancy and resistance mechanisms. Nevertheless, important gaps remain regarding its bioavailability, optimal dosing, and interactions with current therapeutic regimens. Future

studies should therefore focus on detailed pharmacokinetic characterization, validation of molecular targets in clinically relevant models, and investigation of possible synergistic effects with standard prostate cancer therapies to determine whether the pleiotropic actions of carnosol can translate into meaningful clinical benefits.

Rosmarinic acid

In vitro

Treating PC-3 and DU145 cells with rosmarinic acid reduced cancer cell proliferation and suppressed both colony formation and tumor spheroid development, indicating impaired tumorigenic potential. Apoptotic induction was confirmed through increased early and late apoptosis in rosmarinic acid-treated cells. Rosmarinic acid decreased the expression of histone deacetylase (HDAC) 2, a member of the class I HDAC family frequently overexpressed in prostate cancer and associated with suppression of the tumor suppressor protein p53. Down-regulation of HDAC2 by rosmarinic acid resulted in increased p53 expression, accompanied by reduced levels of proliferation-related proteins including cyclin E1, cyclin D1, and proliferating cell nuclear antigen (PCNA), along with up-regulation of the cyclin-dependent kinase inhibitor p21. Furthermore, rosmarinic acid modulated components of the intrinsic mitochondrial apoptotic pathway, as reflected by increased Bax, caspase-3 activation, and cleavage of PARP-1, together with decreased Bcl-2 expression (41)(Table 2).

In brief, evidence regarding the anticancer activity of rosmarinic acid in prostate cancer remains very limited, with only one *in vitro* study currently available (41). Rosmarinic acid may exert anticancer effects in prostate cancer primarily through epigenetic and cell-cycle regulatory mechanisms. Inhibition of HDAC2 appears to be a central event linking rosmarinic acid to the reactivation of the tumor suppressor protein p53 and subsequent up-regulation of the cyclin-dependent kinase inhibitor p21. This pathway suggests that rosmarinic acid may restore tumor-suppressive transcriptional programs that are frequently silenced in prostate cancer. Through this mechanism, HDAC2 inhibition could simultaneously suppress proliferative signaling and promote mitochondrial apoptosis, highlighting a potential role for rosmarinic acid as a natural epigenetic modulator. Such activity is particularly relevant because HDAC overexpression has been associated with aggressive and treatment-resistant prostate tumors, suggesting that compounds targeting this pathway may complement existing therapeutic strategies.

However, the current evidence remains extremely limited, as these findings are derived from a single *in vitro* study using androgen-independent prostate cancer cell lines (PC-3 and DU145). The relatively high concentrations tested (25-300 μM) also raise concerns regarding physiological relevance and potential clinical applicability. Although the study employed several functional assays, including colony and spheroid formation, the lack of *in vivo* validation, pharmacokinetic analysis, and evaluation in normal prostate cells restricts conclusions about therapeutic selectivity and safety. Therefore, while rosmarinic acid shows promise as a modulator of epigenetic and apoptotic pathways in prostate cancer, the very small number of available studies highlights a significant research gap and underscores the need for further mechanistic, *in vivo*, and translational investigations.

Ursolic acid

In vitro

Ursolic acid treatment was assessed in human hormone-refractory prostate cancer PC-3 cells and LNCaP cells, and the obtained data demonstrated that exposure to ursolic acid significantly reduced cell viability and promoted apoptotic cell death in both cell lines, with effective concentrations of approximately 55 μM in PC-3 cells and 45 μM in LNCaP cells. These effects were accompanied by decreased expression of Bcl-2 (42).

The findings showed that ursolic acid significantly reduced cell viability in DU145 cells by promoting apoptotic cell death, accompanied by activation of caspase-3. At the signaling level, ursolic acid selectively activated c-Jun N-terminal kinase (JNK) without noticeably affecting extracellular signal-regulated kinases 1 and 2 (ERK1/2) or p38 mitogen-activated protein kinase (p38 MAPK). Activation of JNK was associated with phosphorylation of Bcl-2 at serine 70 and its subsequent degradation (43).

Another study evaluating the pro-apoptotic effects of ursolic acid in prostate cancer compared its activity in androgen-dependent LNCaP cells and androgen-independent LNCaP-AI cells, which typically exhibit elevated levels of the anti-apoptotic Bcl-2. The results showed that ursolic acid effectively induced apoptosis in both cell types and overcame Bcl-2–associated resistance to cell death in androgen-independent cells. Mechanistic investigations indicated that ursolic acid activated JNK, leading to phosphorylation and degradation of Bcl-2 and subsequent activation of caspase-9, thereby initiating the intrinsic apoptotic pathway (44).

An investigation evaluating the anticancer effects of ursolic acid demonstrated significant growth inhibition in PC-58T/h/SA#4 cells, which are primary malignant tumor-derived human prostate cells. The compound suppressed cell proliferation in both dose- and time-dependent manners and promoted apoptotic cell death. Ursolic acid increased the proportion of cells in the sub-G1 phase, formation of apoptotic bodies, nuclear condensation, and fragmentation of DNA. Treatment with ursolic acid at concentrations exceeding 40 μM markedly elevated the activity of caspase-3, caspase-8, and caspase-9 compared with untreated controls. Additionally, the compound altered the balance of Bax, while reducing Bcl-2. Cleavage of BH3 interacting domain death agonist (Bid) was also observed. Moreover, elevated expression of apoptosis-inducing factor (AIF), a mitochondrial mediator of caspase-independent apoptosis, further confirmed that ursolic acid can induce programmed cell death through both caspase-dependent and caspase-independent pathways (45).

Treating PC-3 cells with ursolic acid reduced cell viability in a dose-dependent manner by inducing apoptosis. Both extrinsic and intrinsic apoptotic pathways were involved, as inhibition of caspase-8 and caspase-9 reduced ursolic acid-induced apoptosis. The compound activated JNK, leading to Bcl-2 phosphorylation and caspase-9 activation, while simultaneously suppressing the Akt pathway, thereby increasing Fas ligand (FasL) expression and triggering death receptor-mediated apoptosis. Furthermore, ursolic acid inhibited the invasive potential of PC-3 cells by down-regulating matrix metalloproteinase-9 (MMP-9) through Akt inhibition (46).

Ursolic acid was evaluated *in vitro* in prostate cancer

cell lines to determine its effect on metastatic signaling pathways. Treatment with ursolic acid resulted in a dose- and time-dependent reduction in C-X-C chemokine receptor type 4 (CXCR4) expression, independent of human epidermal growth factor receptor 2 (HER2) status. The decrease in CXCR4 was not associated with proteasomal or lysosomal degradation, indicating regulation at the transcriptional level. Further analyses demonstrated that ursolic acid suppressed CXCR4 mRNA expression and inhibited the activation of NF-κB. As a consequence, ursolic acid significantly reduced C-X-C motif chemokine ligand 12 (CXCL12)-induced migration and invasion of prostate cancer cells, suggesting an inhibitory effect on metastatic behavior (47).

Investigating the anti-cancer potential of ursolic acid in DU145 and LNCaP cell lines demonstrated that ursolic acid suppressed both constitutive and TNF-α–stimulated activation of NF-κB in a dose-dependent manner. This inhibitory effect occurred through blockade of IKK activation and subsequent reduction in phosphorylation of IκBα and the p65 subunit, leading to decreased NF-κB–dependent transcriptional activity. In addition, ursolic acid reduced constitutive and inducible activation of STAT3 by inhibiting upstream kinases, including Src and Janus kinase 2 (JAK2), and by lowering STAT3-dependent reporter gene activity. The compound also diminished the expression of multiple genes regulated by NF-κB and STAT3 that are associated with cellular proliferation, survival, and angiogenesis, while promoting apoptosis (48).

Another study aimed at developing novel anticancer agents synthesized a series of ursolic acid ((1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid) derivatives bearing a 12-fluoro-13,28β-lactone moiety using the electrophilic fluorination reagent Selectfluor and evaluated their antiproliferative activity in different cancer cell models. Among the synthesized compounds, 2-cyano-3-oxo-12α-fluoro-urs-1-en-13,28β-olide (compound 20) demonstrated strong inhibitory activity against PC-3 cells, exhibiting an IC_{50} of less than 1 μM (49).

Experiments conducted in DU145 prostate cancer cells showed that ursolic acid increased intracellular adenosine triphosphate (ATP) levels and elevated the expression of the purinergic receptor P2Y2 (P2Y2). Activation of this receptor stimulated the Src proto-oncogene tyrosine kinase (Src), which subsequently triggered phosphorylation of p38 MAPK. This signaling cascade resulted in enhanced expression of cyclooxygenase-2 (COX-2), a key enzyme associated with survival signaling that contributed to resistance against UA-induced apoptosis. In addition, the release of arachidonic acid, the substrate required for COX-2 activity, was mediated by calcium-independent phospholipase A2 (iPLA2) and calcium-dependent secretory phospholipase A2 (sPLA2). Evidence also indicated that the initiation of apoptosis following ursolic acid treatment depended on the activation of protein kinase C (PKC) in cells (50).

Another study found that ursolic acid reduced cell viability and exerted antiproliferative activity characterized by arrest of the cell cycle at the G1 phase in PTEN–deficient prostate cancer PC-3 cells. Ursolic acid triggered a pronounced autophagic response, which was evidenced by increased expression of microtubule-associated protein 1 light chain 3-II (LC3-II), a marker of autophagosome formation in mammalian cells, along with enhanced incorporation of monodansylcadaverine into autolysosomes. Further analyses indicated that the autophagic process occurred before apoptosis and was regulated through Beclin-1 and the Akt/mTOR signaling pathway. Furthermore, inhibition of autophagy using 3-methyladenine or small interfering RNA targeting Beclin-1 and autophagy related 5 (Atg5) significantly intensified ursolic acid–induced apoptosis, suggesting that autophagy may function as a protective mechanism that enables PC-3 cells to resist apoptotic cell death (51).

A study exploring new therapeutic strategies for prostate cancer evaluated whether ursolic acid could enhance the antitumor activity of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL is known to selectively trigger apoptosis in many cancer cells with minimal toxicity to normal tissues; however, certain prostate cancer cells exhibit resistance to its apoptotic effects. Experimental findings demonstrated that treatment with ursolic acid increased TRAIL-mediated apoptosis in prostate cancer cells. It also promoted the up-regulation of death receptor 5 (DR5) through a pathway dependent on CHOP, thereby increasing the sensitivity of tumor cells to TRAIL-induced cell death (52).

It has been shown that ursolic acid exhibited cytotoxic activity against PC-3, LNCaP, and DU145 cells, with IC_{50} values of approximately 35 μM in PC-3 cells, 47 μM in LNCaP cells, and 80 μM in DU145 cells. Treatment increased apoptotic features, including ethidium homodimer-positive cells, apoptotic body formation, and dose-dependent accumulation of cells in the sub-G1 phase, indicating enhanced apoptosis in PC-3 cells. Molecular analyses further demonstrated activation of apoptotic signaling, characterized by cleavage of PARP and activation of caspase-9 and caspase-3, accompanied by reduced expression of anti-apoptotic proteins such as B-cell lymphoma 2-like protein 1 (Bcl-XL), Bcl-2, and myeloid cell leukemia 1 (Mcl-1), along with increased expression of Bax. In addition, ursolic acid suppressed components of the Wnt signaling pathway, including Wnt5a/b and β-catenin, while increasing phosphorylation of glycogen synthase kinase 3 beta (GSK3β)(53).

A study evaluating the anticancer activity of ursolic acid reported significant inhibitory effects on prostate cancer cells *in vitro*. Treatment of human prostate cancer cell lines LNCaP and PC-3 with ursolic acid reduced cell proliferation and triggered apoptosis. The induction of programmed cell death was accompanied by decreased expression of several anti-apoptotic proteins, including Bcl-2, Bcl-XL, and survivin, and by activation of caspase-3. Additionally, ursolic acid treatment suppressed the PI3K signaling pathway by reducing PI3K expression and decreasing phosphorylation of Akt and mTOR, indicating disruption of a key survival pathway in prostate cancer cells (54).

Similarly, exposing DU145 cells to ursolic acid inhibited cell proliferation and induced apoptosis. Consistently, ursolic acid treatment increased the activity of caspase-3 and caspase-9. It also promoted the release of cytochrome c from mitochondria into the cytoplasm, suppressed the rho-associated protein kinase (ROCK)/PTEN signaling pathway, and reduced the expression of cofilin-1 (55).

Likewise, another study examined the pro-apoptotic activity of ursolic acid on human prostate cancer LNCaP cells and the underlying molecular mechanisms. The results illustrated that treatment with ursolic acid markedly reduced cell proliferation and promoted apoptosis, as evidenced by increased activities of caspase-3 and caspase-9. Ursolic acid also modulated the ROCK1/PTEN signaling axis, leading to altered expression of cofilin-1 and enhanced release of cytochrome c from mitochondria (56).

Evaluating the effects of ursolic acid on antioxidant signaling in prostate cancer cells demonstrated that treatment with this phytochemical increased the expression of SET domain-containing lysine methyltransferase 7 (SETD7), an epigenetic regulator involved in cellular defense mechanisms. Enhanced SETD7 expression was associated with activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway and increased transcription of downstream antioxidant genes, including NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione S-transferase theta 2 (GSTT2). Epigenetic analysis further showed that ursolic acid treatment elevated histone H3 lysine 4 monomethylation (H3K4me1) at the promoter regions of Nrf2 and GSTT2, suggesting an epigenetic mechanism underlying this regulatory effect. Through these changes, ursolic acid contributed to strengthening the Nrf2/antioxidant response element (ARE) pathway, which was associated with enhanced cellular protection against oxidative stress–induced DNA damage in prostate cancer cells (57).

An investigation exploring the mechanisms responsible for the anticancer effects of ursolic acid examined its influence on nuclear receptor–mediated signaling pathways in prostate cancer cells. The findings revealed that ursolic acid significantly inhibited the transactivation function of retinoic acid receptor-related orphan receptor gamma (RORγ), a nuclear receptor known to contribute to tumor progression and the regulation of oncogenic signaling networks. In prostate cancer models, inhibition of RORγ by ursolic acid led to a reduction in androgen receptor expression and attenuation of androgen receptor signaling, which plays an essential role in prostate cancer development and progression (58).

Ursolic acid exerted inhibitory effects on benign prostatic hyperplasia cells. Treatment with ursolic acid significantly reduced cellular proliferation while promoting apoptosis. These effects were associated with induction of S-phase cell-cycle arrest, which contributed to suppression of benign prostatic hyperplasia cell growth (59).

Mechanistic evaluation of ursolic acid was further conducted in mouse and human prostate cancer cell lines to clarify its cellular effects. Ursolic acid exposure affected regulators of cell cycle progression, including decreased expression of cyclin D1, cyclin-dependent kinase (CDK)4, and reduced phosphorylation of retinoblastoma protein. Further cellular effects of ursolic acid included modulation of proteins involved in stress responses and apoptosis. The treatment was associated with increased expression of activating transcription factor 4 (ATF4) and CHOP, indicating activation of stress-related pathways linked to the unfolded protein response. In addition, ursolic acid reduced levels of anti-apoptotic proteins such as Mcl-1 and Bcl-2 in prostate cancer cells (60)(Table 3).

Research exploring the anticancer activity of ursolic acid examined the compound and several of its semi-synthetic derivatives obtained after isolation from *Mimusops caffra*. Following the identification of ursolic acid and its structural isomer oleanolic acid, several modified derivatives were generated, including 3-O-acetyl ursolic acid, ursolic-28-methylate, and 3-acetylursolic-methylate, to evaluate their potential anticancer properties further. The cytotoxic activities of these compounds were examined *in vitro* against PC3 cells. The findings indicated that the methylated derivative ursolic-28-methylate exhibited the strongest cytotoxic activity against the cancer cell line while demonstrating minimal toxicity toward non-cancerous cells. In contrast, 3-O-acetyl ursolic acid displayed weaker antiproliferative activity compared with the parent compound ursolic acid. These results suggest that structural modification of ursolic acid, particularly methylation at the C-28 position, may enhance its anticancer efficacy (61).

In vivo

Exploring the effects of ursolic acid using the TRAMP model disclosed that prostate tissues from ursolic acid-treated animals exhibited reduced CXCR4 expression (48).

An experimental investigation assessed the chemopreventive effects of ursolic acid on prostate tumor development using the TRAMP mouse model—dietary administration of ursolic acid produced stage-specific protective effects during prostate tumorigenesis. Feeding mice with an ursolic acid-enriched diet for eight weeks during the early phase (weeks 4-12) delayed the appearance of prostatic intraepithelial neoplasia, a precancerous lesion. In a later stage (weeks 12-18), ursolic acid supplementation for six weeks suppressed the progression of prostatic intraepithelial neoplasia to adenocarcinoma. Furthermore, prolonged administration of ursolic acid for twelve weeks (weeks 24-36) markedly reduced tumor growth, improved overall survival, and did not significantly affect body weight. Ursolic acid treatment inhibited the activation of several pro-inflammatory and oncogenic signaling pathways in dorsolateral prostate tissues, including Akt, NF-κB, STAT3, and IKKα/β phosphorylation, which was associated with decreased serum levels of interleukin-6 (IL-6) and TNF-α. Further experiments revealed reduced expression of cyclin D1 and COX-2, along with increased levels of caspase-3. Additionally, measurable nanogram concentrations of ursolic acid were detected in mouse serum, demonstrating effective gastrointestinal absorption (62).

The administration of ursolic acid significantly inhibited tumor growth in LNCaP xenografts established in athymic nude mice. Tumor tissues obtained from ursolic acid-treated animals displayed reduced cellular proliferation and increased apoptotic activity. Furthermore, molecular analyses of the xenograft tissues demonstrated decreased expression of downstream components of the PI3K pathway, particularly phosphorylated Akt (p-Akt) and phosphorylated mechanistic target of rapamycin (p-mTOR) (54).

Evaluation of ursolic acid in an *in vivo* xenograft model provided evidence for its antitumor activity against prostate cancer. Treatment with ursolic acid led to a significant reduction in tumor growth in immunodeficient mice bearing transplanted human VCaP-Luc prostate cancer cells, indicating its capacity to restrain tumor progression.

Table 3. Biological effects of ursolic acid on prostate cancer cells and animal models

Compound	Study design	Doses/Duration	Results	Ref.
		In vitro		
Ursolic acid	PC-3 cells and LNCaP cells	1-100 µM	↑ Apoptosis ↓ Cell viability, Bcl-2 expression	(39)
Ursolic acid and its ester derivatives	DU145 cells	-	↓ MMP2 and MMP-9 activity	(62)
Ursolic acid	PTEN–deficient PC-3 cells	0-40 µM	↑ Antiproliferative activity, cell cycle arrest at the G1 phase, autophagic response, LC3-II expression ↓ Cell viability	(46)
Ursolic acid	PC-3, LNCaP, and DU145 cells	0-100 µM	↑ Cytotoxic activity, apoptotic features, ethidium homodimer-positive cells, apoptotic body formation, cells in the sub-G1 phase, PARP cleavage, activation of caspase-9 and caspase-3, Bax expression, GSK3β phosphorylation ↓ Expression of Bcl-XL, Bcl-2, Mcl-1, Wnt5a/b, β-catenin	(48)
Ursolic acid	DU145 cells	0, 10, 20 and 40 µM	↑ Apoptosis, activity of caspase-3 and caspase-9, cytochrome c release from mitochondria ↓ Cell proliferation, ROCK/PTEN signaling pathway, cofilin-1 expression	(49)
Ursolic acid	LNCaP cells	0, 10, 20 and 40 µM	↑ Apoptosis, activity of caspase-3 and caspase-9, p-PTEN, cofilin-1 ↓ Cell proliferation, ROCK1	(50)
Ursolic acid	LNCaP and PC3 cells	5 and 20 µM	↑ SETD7 expression, Nrf2 signaling pathway, transcription of NQO1 and GSTT2, H3K4me1 at the promoter regions of Nrf2 and GSTT2, ARE pathway	(51)
Ursolic acid	C4-2B, 22RV1, LNCaP, PC3 and cells	0-10 µM	↓ Transactivation function of RORγ, androgen receptor expression, androgen receptor signaling	(52)
Ursolic acid	Benign prostatic hyperplasia cells	0-30 µmol/l	↑ Apoptosis, S-phase cell-cycle arrest ↓ Cellular proliferation, hyperplasia	(59)
Ursolic acid	HMVP2 and LNCaP cells	10 uM	↑ Expression of ATF4 and CHOP ↓ Expression of cyclin D1, CDK4, retinoblastoma protein phosphorylation, Mcl-1, Bcl-2	(53)
		In vivo		
Ursolic acid	Nude mice	200 mg/kg, t weeks	↓ DU145 tumor growth	(43)
Ursolic acid	Inbred male TRAMP mice	0.1% of diet, p.o.	↑ Survival, caspase-3 level ↓ Appearance and progression of prostate intraepithelial neoplasia, tumor growth, Akt, NF-κB, STAT3, and IKKα/β phosphorylation, IL-6, TNF-α, expression of cyclin D1 and COX-2	(55)
Ursolic acid	Male NCr immunodeficient mice	0.1% of diet, 8 weeks, p.o.	↓ Tumor growth	(57)
Ursolic acid	PTEN knockout mice	0.1% of diet, 6 weeks, p.o.	↓ Tumor development, HAS3, MSX1expression, cancer-associated metabolic disturbances	(58)
Ursolic acid	Castrated rats	5 mg/kg, p.o.	↑ Regular glandular lumen, well-organized acinar structures lined with single-layer columnar epithelial cells ↓ Prostate weight and volume, prostate index, stromal proliferation, inflammatory cell infiltration	(59)
Ursolic acid	HiMyc transgenic mice and PTEN knockout mice	0.2% of diet, 5–6 weeks, p.o.	↑ PTEN, p27, E-cadherin ↓ Phosphorylation of Akt, STAT3 levels, cyclin A, MTA1, SOX4, TWIST1	(53)

Akt: protein kinase B; ARE: antioxidant response element; ATF4: activating transcription factor 4; Bax: B-cell lymphoma-2–associated X protein; Bcl-2: B-cell lymphoma 2; Bcl-XL: B-cell lymphoma 2-like protein 1; CDK4: cyclin-dependent kinase 4; CHOP: CCAAT/enhancer-binding protein homologous protein; COX-2: cyclooxygenase-2; GSK3β: glycogen synthase kinase 3 beta; GSTT2: glutathione S-transferase theta 2; H3K4me1: histone H3 lysine 4 monomethylation; IKK: inhibitor of κB kinase; IL-6: interleukin-6; LC3-II: microtubule-associated protein 1 light chain 3-II; Mcl-1: myeloid cell leukemia 1; MMP: matrix metalloproteinase; MSX1: msh homeobox 1; NF-κB: nuclear factor kappa B; NQO1: NAD(P)H quinone dehydrogenase 1; Nrf2: nuclear factor erythroid 2-related factor 2; p.o.: per os (oral administration); PARP: poly(adenosine diphosphate-ribose) polymerase; PTEN: phosphatase and tensin homolog; Ref.: reference; ROCK: Rho-associated protein kinase; RORγ: retinoic acid receptor-related orphan receptor gamma; STAT3: signal transducer and activator of transcription 3; TNF-α: tumor necrosis factor alpha

The antitumor effect appears to be linked, at least in part, to ursolic acid-induced alterations in cellular metabolism, particularly changes involving S-adenosylmethionine. These metabolic shifts may influence epigenetic regulation, including modifications in DNA methylation, which in turn could affect the expression of genes involved in cancer-related signaling pathways (63).

Furthermore, ursolic acid treatment was found to attenuate prostate tumor development induced by PTEN knockout at different stages by reducing the number and magnitude of differentially methylated regions associated with PTEN deficiency. In parallel, ursolic acid counteracted PTEN knockout–induced changes in gene expression, including the overexpression of several prostate cancer–related oncogenes such as complement factor H (CFH), hyaluronan synthase 3 (HAS3), and msh

homeobox 1 (MSX1). The expression of genes including the tumor suppressor 3-hydroxybutyrate dehydrogenase type 2 (BDH2) and oncogenes such as EPH receptor A5 (EPHA5), interferon-stimulated gene 15 (ISG15), and nitric oxide synthase 2 (NOS2) was associated with alterations in promoter CpG methylation during early stages of tumorigenesis, suggesting that ursolic acid may influence gene activity through epigenetic modulation. In addition, ursolic acid attenuated PTEN knockout–associated metabolic disturbances, including alterations in purine metabolism, glycolysis, and gluconeogenesis pathways, as well as levels of metabolites linked to epigenetic regulation such as pyruvate and lactate (64).

In an experimental rat model of benign prostatic hyperplasia, animals were divided into five groups: a control group, a benign prostatic hyperplasia model group, and three treatment groups receiving rescinnamine, lumicolchicine, or ursolic acid. Following four weeks of treatment, ursolic acid markedly decreased prostate weight and volume, resulting in a significant reduction in the prostate index compared with the other treatment groups (rescinnamine and lumicolchicine), indicating the strongest therapeutic effect. Histopathological assessment further showed that ursolic acid substantially improved benign prostatic hyperplasia-related prostate alterations, characterized by a more regular glandular lumen, well-organized acinar structures lined with single-layer columnar epithelial cells, limited stromal proliferation, and minimal inflammatory cell infiltration (59).

In vivo evaluation of ursolic acid was performed in the HiMyc transgenic mouse model of prostate cancer. Mice fed a diet containing 0.2% ursolic acid from 5-6 weeks of age until six months showed good tolerance, with no significant differences in food intake or body weight compared with controls. Histopathological assessment of ventral prostate tissues revealed the presence of in situ and locally invasive adenocarcinoma, indicating ongoing tumor development in this model. Ursolic acid was associated with increased PTEN protein levels and reduced phosphorylation of Akt and STAT3. It also altered regulators of cell cycle progression, including decreased cyclin A and increased p27, alongside changes in epithelial–mesenchymal transition markers such as increased E-cadherin and reduced MTA1, SOX4, and TWIST1 (60).

Across the available studies, ursolic acid consistently demonstrates multi-targeted antitumor activity in prostate cancer models, with its protective role largely emerging from coordinated modulation of apoptotic, inflammatory, metabolic, and transcriptional signaling networks (Figure 5). Rather than acting through a single dominant pathway, ursolic acid appears to reprogram tumor cell survival circuits by simultaneously suppressing pro-survival signaling and activating stress- and death-associated pathways. A central mechanistic theme involves the disruption of mitochondrial integrity and activation of the intrinsic apoptotic machinery. Multiple studies show that ursolic acid decreases anti-apoptotic proteins such as Bcl-2, Bcl-XL, and Mcl-1 while increasing pro-apoptotic mediators including Bax and caspase-3/9 activation (42, 53, 54). Systematically, this effect is closely linked to JNK activation, which promotes phosphorylation-dependent degradation of Bcl-2 and facilitates mitochondrial cytochrome-c release (43, 44, 46). The mitochondrial destabilization is reinforced

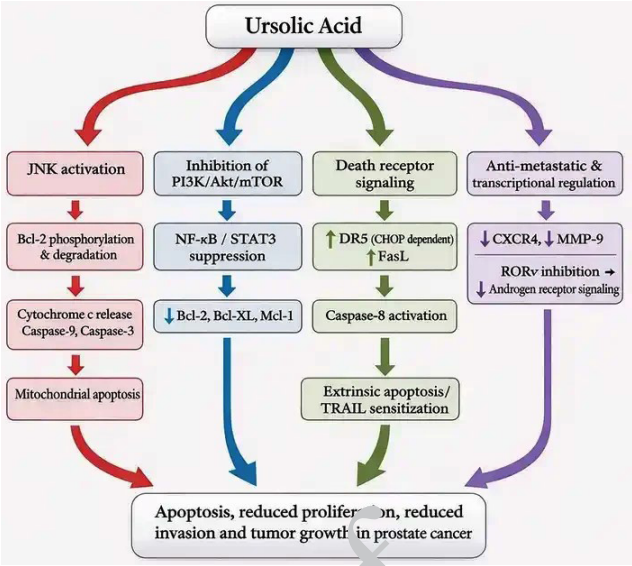

Figure 5. Mechanistic overview of the anti cancer effects of ursolic acid in prostate cancer
Ursolic acid modulates multiple sig pathways that collectively suppress prostate cancer progression. It activates the JNK pathway, promoting Bcl-2 phosphorylation and degradation, which trigg s cytochrome c release, activation of caspase-9 and caspase-3, and mitoch drial apoptosis. It also inhibits the PI3K/Akt/mTOR pathway, suppressing NF-κB an STAT3 signaling and down-regulating anti-apoptotic proteins, including Bcl-2, Bcl-XL, and Mcl-1. In parallel, ursolic acid enhances death receptor signal g by increasing DR5 (via CHOP-dependent mechanisms) and Fas expression, l ding to caspase-8 activation and extrinsic apoptosis/ TRAIL sen itization. Additionally, it exerts anti-metastatic effects by reducing CXCR4 and MMP-9 expression and inhibiting RORγ-mediated androgen receptor signaling. These c ordinated effects ultimately result in increased apoptosis, reduced prol tic de reased invasion, and suppression of tumor growth in prostate cancer

by simultaneous suppression of major oncogenic signaling axes such as PI3K/Akt/mTOR, NF-κB, and STAT3, pathways that normally maintain proliferation, survival, and inflammatory signaling in prostate tumors (48, 54). By targeting these interrelated networks, ursolic acid appears to shift the cellular equilibrium from survival toward programmed cell death.

Beyond its pro-apoptotic effects, accumulating evidence indicates that ursolic acid may also influence higher-level regulatory processes in tumor biology, including transcriptional regulation, epigenetic modifications, and cellular metabolic pathways. Inhibition of RORγ and subsequent suppression of androgen receptor signaling indicates that ursolic acid may interfere with one of the central drivers of prostate cancer progression (58). Additionally, epigenetic studies demonstrate that ursolic acid can modify histone methylation patterns and influence SETD7-dependent activation of the Nrf2 antioxidant pathway (57), while *in vivo* work indicates that ursolic acid alters DNA methylation patterns and metabolic intermediates associated with epigenetic regulation (64). Taken together, these findings suggest that ursolic acid may function as a metabolic–epigenetic regulator that can influence gene expression patterns and thereby reshape the transcriptional landscape of prostate cancer cells. Such a mechanism may partly explain the ability of ursolic acid to simultaneously suppress oncogenic signaling, inflammation, and metastatic potential, including down-regulation of CXCR4-mediated migration (48) and MMP-dependent invasion (65). Conceptually, this integrated activity supports the idea that ursolic acid does not simply induce

cytotoxicity but may push malignant cells toward a less aggressive phenotype by restoring regulatory balance across multiple signaling layers.

Nevertheless, some mechanistic findings reveal important biological complexities. For example, although ursolic acid generally activates apoptotic pathways, certain studies demonstrate activation of survival-associated signals such as P2Y2/Src/p38 MAPK and COX-2 expression, which may partially counteract ursolic acid-induced apoptosis (50). Similarly, ursolic acid-induced autophagy appears to function as a protective response in some prostate cancer cells, where inhibition of autophagy enhances ursolic acid-mediated apoptosis (51). These apparently contradictory findings may reflect adaptive stress responses triggered by ursolic acid exposure. From a therapeutic perspective, this suggests that combining ursolic acid with inhibitors of autophagy or inflammatory signaling might enhance its anticancer efficacy. Such combinatorial strategies could potentially convert compensatory survival responses into vulnerabilities within tumor cells.

Although the experimental evidence supporting the anticancer activity of ursolic acid is substantial, several methodological limitations should be considered when interpreting these findings. Many *in vitro* studies employ relatively high concentrations of ursolic acid (often 40-100 μM) to achieve cytotoxic effects, raising questions about whether comparable levels can be achieved in human tissues following oral administration. Differences in sensitivity among prostate cancer cell lines also complicate interpretation, as androgen-dependent and androgen-independent models exhibit distinct molecular characteristics. Furthermore, several studies rely heavily on biochemical markers of apoptosis without extensive validation using complementary approaches such as genetic pathway inhibition or long-term clonogenic assays. The *in vivo* studies provide stronger translational relevance, demonstrating reduced tumor growth and modulation of oncogenic signaling in xenograft, TRAMP, and transgenic mouse models (60, 62, 63). However, these models still lack the full complexity of human prostate cancer progression, particularly regarding tumor heterogeneity, immune interactions, and long-term pharmacokinetics.

Despite these limitations, the collective findings provide several strengths. The reproducibility of ursolic acid-induced apoptosis across multiple prostate cancer cell lines and animal models supports the strength of its anticancer activity. Moreover, the convergence of evidence pointing toward inhibition of key oncogenic pathways, PI3K/Akt, NF-κB, STAT3, androgen receptor signaling, and CXCR4, suggests that ursolic acid may function as a pleiotropic signaling modulator rather than a single-target agent. This broad mode of action is especially relevant in prostate cancer, where the presence of multiple overlapping signaling pathways often allows tumor cells to bypass targeted therapies and develop resistance.

Translation into clinical practice remains an important challenge. One major limitation is the relatively low bioavailability of ursolic acid, which may restrict systemic exposure after oral intake. Structural modification studies showing enhanced potency of certain ursolic acid derivatives, including fluorinated or methylated analogs with sub-micromolar activity (49, 61), highlight a promising direction for improving pharmacological performance. Future therapeutic strategies might therefore focus on optimized ursolic acid derivatives, targeted delivery systems, or rational drug combinations with agents such as TRAIL or PI3K inhibitors. From a conceptual standpoint, ursolic acid may be particularly valuable as a chemopreventive or adjuvant compound that targets early tumorigenic signaling, inflammation, and metabolic dysregulation simultaneously. Such multi-layered modulation could theoretically delay progression from premalignant lesions to invasive prostate cancer, as suggested by chemoprevention data in transgenic mouse models.

Overall, the current body of evidence indicates that ursolic acid exerts protective effects against prostate cancer through a complex network of biochemical mechanisms involving apoptosis induction, suppression of oncogenic signaling, modulation of epigenetic and metabolic pathways, and inhibition of metastatic processes. While promising, further research integrating pharmacokinetic studies, clinically relevant dosing strategies, and well-designed clinical trials will be essential to determine whether these mechanistic insights can be effectively translated into therapeutic or preventive interventions in humans.

Convergence and specificity in signaling pathways

When the available evidence on rosemary extract and its main components is considered collectively, an important pattern becomes apparent. Despite their structural diversity, many of these compounds converge on a limited number of key oncogenic pathways that regulate prostate cancer cell survival, proliferation, and stress responses. At the same time, each compound exhibits distinct secondary mechanisms that influence different biological layers of tumor regulation. This combination of shared targets and compound-specific actions creates both mechanistic convergence and functional divergence among rosemary-derived compounds.

One of the most consistent points of convergence involves inhibition of the PI3K/Akt/mTOR signaling axis, a central pathway controlling tumor growth, metabolism, and survival in prostate cancer. Several rosemary-derived compounds, including rosemary extracts, carnosic acid, carnosol, and ursolic acid, have been shown to suppress Akt signaling or its downstream pathways. Through this mechanism, these compounds reduce mTOR-dependent anabolic growth, weaken NF-κB-mediated survival signaling, and promote apoptotic responses. Such convergence suggests that rosemary phytochemicals may destabilize a central regulatory core that integrates proliferative and metabolic signals in prostate tumor cells.

Another shared mechanism is the induction of cellular stress responses that culminate in apoptosis, although the initiating pathways differ between compounds. Ursolic acid and betulinic acid primarily induce mitochondrial apoptosis through modulation of Bcl-2 family proteins and caspase activation. In contrast, carnosic acid and carnosol frequently promote broader stress responses, including metabolic stress through AMPK activation and endoplasmic reticulum stress. Rosemary extracts likely reflect a combination of these mechanisms due to the synergistic activity of multiple phenolic and terpenoid constituents.

Despite these shared effects, important mechanistic differences also exist. Betulinic acid uniquely interferes with the ubiquitin–proteasome system and transcription factor stability. In contrast, ursolic acid appears to influence

Table 4. Major mechanistic convergence across rosemary-derived compounds in prostate cancer

Shared biological process	Key molecular targets/pathways	Compounds involved	Functional consequence
Survival signaling inhibition	PI3K/Akt/mTOR pathway	Rosemary extract, carnosic acid, carnosol, ursolic acid	Reduced proliferation and anabolic growth
Apoptosis induction	Bax/Bcl-2 modulation, caspase activation, mitochondrial dysfunction	Betulinic acid, carnosic acid, carnosol, rosmarinic acid, ursolic acid	Activation of intrinsic apoptotic pathways
Inflammatory signaling suppression	NF-κB and STAT3 inhibition	Betulinic acid, carnosic acid, ursolic acid	Reduced pro-survival and inflammatory gene expression
Metabolic stress induction	AMPK activation and mTOR inhibition	Carnosic acid, carnosol	Energy stress and suppression of biosynthetic pathways

Akt: protein kinase B; AMPK: adenosine monophosphate-activated protein kinase; Bax: B-cell lymphoma-2–associated X protein; Bcl-2: B-cell lymphoma-2; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor-kappa B; PI3K: phosphatidylinositol 3-kinase; STAT3: signal transducer and activator of transcription 3

transcriptional and epigenetic regulation, including changes in histone methylation and DNA methylation. Carnosic acid shows distinctive activity in promoting androgen receptor degradation through endoplasmic reticulum-stress-related pathways, whereas rosmarinic acid primarily acts as an epigenetic modulator through inhibition of HDAC2. These differences indicate that rosemary phytochemicals influence multiple layers of tumor biology, including signaling pathways, protein homeostasis, and chromatin regulation.

Generally, this pattern of convergence and divergence (Tables 4 and 5) supports the view that rosemary compounds act as multi-target network modulators rather than single-pathway inhibitors. By simultaneously affecting several interconnected signaling systems, these phytochemicals may reduce the likelihood of compensatory pathway activation, which often drives therapeutic resistance in prostate cancer. Consequently, their greatest therapeutic value may lie in combination or sensitization strategies rather than as standalone treatments.

Therapy resistance, lineage plasticity, and immunological implications

The anticancer effects of rosemary and its major bioactive constituents, including carnosic acid, carnosol, rosmarinic acid, ursolic acid, and betulinic acid, have been primarily investigated in relation to intracellular signaling pathways regulating proliferation, apoptosis, oxidative stress, and inflammation. However, several clinically relevant aspects of advanced prostate cancer biology remain underexplored in the current literature examining rosemary-derived compounds. In particular, the potential relationship between rosemary bioactives and mechanisms of therapeutic resistance, lineage plasticity, and tumor–immune interactions warrants further consideration.

One important mechanism of resistance in advanced prostate cancer involves androgen receptor splice variants (66), particularly androgen receptor splice variant-7 (AR-V7)(67, 68). Unlike the full-length androgen receptor, AR-V7 lacks the ligand-binding domain targeted by androgen deprivation therapies (69, 70) and next-generation anti-androgens such as enzalutamide and abiraterone (71). As a result, AR-V7 remains constitutively active and can drive persistent androgen receptor signaling despite pharmacological inhibition of androgen production or androgen receptor binding (72, 73). Detection of AR-V7 in circulating tumor cells has been associated with poor

Table 5. Key mechanistic divergence among rosemary compounds

Compound	Distinct molecular mechanisms	Biological implications
Rosemary extract	Polypharmacological suppression of Akt signaling through multiple phenolic and terpenoid constituents	Broad pathway modulation and potential therapeutic sensitization
Betulinic acid	Proteasome-related degradation of Sp1/Sp3/Sp4; inhibition of deubiquitinases; ROS-mediated DNA damage	Anti-angiogenic signaling and disruption of transcription factor stability
Carnosic acid	Endoplasmic reticulum stress induction; androgen receptor degradation; AMPK-mediated metabolic stress	Direct interference with androgen signaling and tumor metabolic adaptation
Carnosol	AMPK activation with suppression of PI3K/Akt and Hedgehog/Gli1 signaling	Cell-cycle arrest and inhibition of stemness-related pathways
Rosmarinic acid	HDAC2 inhibition leading to p53/p21 activation	Epigenetic reactivation of tumor-suppressor transcription programs
Ursolic acid	Epigenetic and transcriptional modulation (RORγ inhibition, SETD7/Nrf2 activation); suppression of CXCR4 and metastasis pathways	Broad regulation of gene expression, inflammation, and metastatic potential

Akt: protein kinase B; AMPK: adenosine monophosphate-activated protein kinase; CXCR4: C-X-C chemokine receptor type 4; DNA: deoxyribonucleic acid; Gli1: glioma-associated oncogene homolog 1; Nrf2: nuclear factor erythroid 2–related factor 2; PI3K: phosphatidylinositol 3-kinase; RORγ: retinoic acid receptor-related orphan receptor gamma; ROS: Reactive oxygen species; SETD7: SET domain-containing lysine methyltransferase 7

response to androgen receptor-targeted therapies and shorter progression-free survival in patients with metastatic castration-resistant prostate cancer (74, 75). Because several rosemary-derived compounds have been reported to influence androgen receptor signaling pathways in prostate cancer models (28, 35, 38, 58), the possibility that these phytochemicals may also affect androgen receptor splice variant expression or activity represents an important but largely unexplored research area. Future studies should therefore examine whether rosemary extracts or individual constituents can modulate AR-V7 expression, nuclear localization, transcriptional activity, or downstream gene programs associated with therapy resistance.

Another critical mechanism of disease progression in advanced prostate cancer is neuroendocrine differentiation, particularly in the context of treatment-emergent neuroendocrine prostate cancer (76, 77). This aggressive subtype frequently arises following prolonged suppression of androgen receptor signaling (78). It is characterized by loss of androgen receptor dependence (79), reduced prostate-specific antigen production (80), and expression of neuroendocrine markers such as chromogranin A and synaptophysin (81, 82). The development of neuroendocrine prostate cancer is increasingly understood as a form of lineage plasticity, driven by genomic alterations such as RB1 and TP53 loss and epigenetic reprogramming that enables tumor cells to escape AR-targeted therapies (83, 84). Although the studies reviewed in the present work demonstrate that rosemary constituents can influence pathways such as PI3K/Akt/mTOR (20, 37, 38), NF-κB (26, 29), and oxidative stress responses (24), their potential effects on lineage plasticity and neuroendocrine differentiation have not yet been systematically investigated. Evaluating whether rosemary-derived compounds can influence neuroendocrine marker expression or prevent lineage switching in prostate cancer models could therefore represent a valuable direction for future research.

In addition to therapy resistance and lineage plasticity, increasing attention has been directed toward the tumor immune microenvironment in prostate cancer. Compared with several other malignancies, prostate tumors are often considered immunologically "cold" (85), characterized by relatively low T-cell infiltration (86) and an immunosuppressive microenvironment dominated by regulatory T cells (87), myeloid-derived suppressor cells (88), and tumor-associated macrophages (89, 90). These features are thought to contribute to the generally modest response rates observed with immune checkpoint inhibitors in unselected prostate cancer populations. Nevertheless, checkpoint blockade therapies such as pembrolizumab have demonstrated clinical activity in molecularly defined subgroups, including tumors with microsatellite instability or high tumor mutational burden (91, 92).

Remarkably, many phytochemicals present in rosemary possess well-documented anti-inflammatory and immunomodulatory properties. Compounds such as carnosic acid, carnosol, and rosmarinic acid have been shown in experimental systems to regulate inflammatory mediators and signaling pathways, including STAT3 (27, 48), COX-2 (50, 62), and cytokine networks (26, 62), that are closely linked to tumor-associated inflammation. Although direct evidence connecting rosemary-derived compounds with modulation of the prostate tumor immune microenvironment remains limited, these observations raise the possibility that such phytochemicals could indirectly influence immune responses within the tumor milieu. Future investigations integrating prostate cancer models with immune components, such as co-culture systems or immunocompetent animal models, may help clarify whether rosemary-derived compounds could enhance antitumor immunity or potentially improve the efficacy of emerging immunotherapeutic strategies.

Collectively, these considerations highlight that while rosemary and its constituents have demonstrated promising anticancer activity in preclinical models, their potential roles in therapy resistance, lineage plasticity, and immune modulation remain insufficiently explored. Addressing these aspects may provide a more comprehensive understanding of how rosemary-derived compounds could be integrated into future strategies for the prevention or treatment of advanced prostate cancer.

State of the art and future perspectives

Current research on rosemary and its main components highlights their growing potential as multi-target modulators in prostate cancer. Experimental evidence accumulated over the past decades demonstrates that rosemary extracts and compounds such as betulinic acid, carnosic acid, carnosol, rosmarinic acid, and ursolic acid can influence several key oncogenic processes, including cell proliferation, apoptosis, inflammation, oxidative stress responses, androgen receptor signaling, and metabolic regulation. Many of these effects converge on major regulatory pathways involved in prostate cancer progression, particularly PI3K/Akt/mTOR, NF-κB, STAT3, and AMPK signaling networks. Through modulation of these pathways, rosemary-derived compounds can impair tumor cell survival, promote programmed cell death, and reduce inflammatory and angiogenic signaling.

Despite these promising findings, the field remains largely dominated by *in vitro* studies and preclinical models, with relatively limited translational and clinical investigation. Most mechanistic data have been generated using prostate cancer cell lines, including androgen-sensitive and androgen-independent models, which have helped clarify the molecular targets of rosemary phytochemicals. Animal studies have further suggested potential antitumor activity and chemopreventive properties, supporting the concept that rosemary compounds may modulate tumor growth and progression. However, clinical evidence remains scarce, and the therapeutic relevance of these findings in human prostate cancer has yet to be firmly established.

Another important challenge concerns the bioavailability and pharmacokinetics of rosemary phytochemicals. Many of these compounds exhibit limited solubility, rapid metabolism, or low systemic bioavailability, which may restrict their effectiveness *in vivo*. Consequently, recent research has begun exploring strategies to improve their stability and delivery, including nanoformulations, encapsulation systems, and combination approaches with other bioactive compounds or conventional therapies.

From a therapeutic perspective, the multi-target nature of rosemary constituents may represent a significant advantage. Complex signaling networks with substantial redundancy and cross-talk between pathways drive prostate cancer progression. Agents capable of simultaneously modulating multiple signaling nodes may therefore reduce

the likelihood of compensatory resistance mechanisms. In this context, rosemary phytochemicals are increasingly being investigated as potential adjuvant or sensitizing agents that could enhance the effectiveness of established treatments such as androgen deprivation therapy, chemotherapy, or targeted inhibitors.

Future research should therefore focus on several key directions. These include deeper characterization of the molecular interactions between rosemary compounds and major oncogenic signaling networks, evaluation of synergistic effects among different phytochemicals, and the development of improved delivery systems to enhance bioavailability. Importantly, well-designed *in vivo* studies and clinical trials will be essential to determine the safety, pharmacokinetics, and therapeutic efficacy of rosemary-derived compounds in prostate cancer patients.

Overall, the current state of research suggests that rosemary and its main components represent promising candidates for further investigation in prostate cancer prevention and therapy. However, bridging the gap between mechanistic insights and clinical application will require integrated efforts combining molecular studies, translational research, and clinical evaluation.

Safety aspects and interaction considerations

Rosemary and its main components are generally considered safe when consumed at dietary levels (10), and several studies suggest a favorable toxicity profile in experimental models. However, the pharmacological concentrations often used in experimental studies may exceed typical dietary exposure, and the safety of long-term supplementation with purified compounds remains insufficiently characterized. In addition, certain rosemary constituents may influence drug-metabolizing enzymes and cellular signaling pathways, raising the possibility of interactions with conventional therapies. Although current evidence suggests relatively low toxicity, further studies are required to define better the pharmacokinetic properties, safe dosage ranges, and potential interactions with anticancer drugs.

Clinical translation challenges

Although numerous preclinical studies highlight the anticancer potential of rosemary and its major phytochemicals, several challenges must be addressed before these compounds can be translated into clinical applications for prostate cancer management.

One of the primary limitations is the relatively low bioavailability of several rosemary-derived compounds. Polyphenols such as rosmarinic acid and diterpenes including carnosic acid and carnosol undergo extensive metabolism in the gastrointestinal tract, which may reduce their systemic concentrations after oral administration (93, 94). Rapid phase II metabolism, including glucuronidation and sulfation, can limit their effective plasma levels and tissue distribution (95). In addition, their lipophilic nature and variable stability may influence absorption and pharmacokinetic behavior (96, 97). Strategies such as nano-formulations, lipid-based carriers, and encapsulation systems have been proposed to improve solubility, stability, and bioavailability of these phytochemicals (98, 99).

Another important aspect concerns the translation of experimental doses used *in vitro* and in animal models into clinically relevant human equivalent doses. Many experimental studies employ concentrations that may not be readily achievable through dietary intake or conventional supplementation. Standard interspecies dose conversion approaches based on body surface area are often used to estimate human equivalent doses from animal experiments. However, these calculations remain approximate and may not accurately reflect human pharmacokinetics, metabolism, or tissue accumulation. Therefore, well-designed pharmacokinetic and dose-escalation studies are necessary to determine safe and therapeutically effective exposure levels in humans.

Potential herb–drug interactions also represent a significant consideration for clinical use. Rosemary and several of its constituents have been reported to interact with cytochrome P450 (CYP450) enzymes (100, 101), which are responsible for the metabolism of many pharmaceutical agents. Experimental evidence suggests that rosemary extracts and compounds such as carnosic acid and rosmarinic acid may modulate CYP isoforms including CYP3A4, CYP2C9, and CYP2D6 (100, 102). Because many drugs used in prostate cancer management, such as anti-androgens, chemotherapeutic agents, and supportive medications, are metabolized through these pathways (103-105), co-administration with rosemary-derived products could theoretically alter drug pharmacokinetics, efficacy, or toxicity. Although clinical data on these interactions remain limited, careful evaluation and monitoring are recommended when herbal supplements are used alongside conventional therapies.

Finally, variability in plant sources, extraction methods, and phytochemical composition may affect reproducibility and clinical translation. Standardization of rosemary extracts and rigorous quality control are essential to ensure consistent concentrations of bioactive compounds in future clinical investigations.

In brief, addressing issues related to bioavailability, dose translation, pharmacokinetics, and potential herb–drug interactions will be critical to advancing rosemary-derived phytochemicals from experimental models to evidence-based clinical applications for prostate cancer.

Limitations and potential sources of bias

Although this narrative review followed a structured literature search strategy similar to those used in systematic reviews, several limitations should be considered. Study selection and synthesis were ultimately performed narratively, which may introduce some degree of interpretative bias. In addition, the available evidence on rosemary compounds in prostate cancer is largely derived from *in vitro* experiments and preclinical models, with limited clinical data. Variability in experimental conditions, compound concentrations, and extraction methods across studies may also affect the comparability of findings.

Another potential limitation is publication bias, in which studies reporting positive or significant biological effects are more likely to be published than those with neutral or negative results. This tendency may overestimate the anticancer potential of rosemary phytochemicals in the current literature. Therefore, while the available data suggest promising biological activity, the conclusions should be interpreted cautiously until further well-designed *in vivo* studies and clinical investigations become available.

Despite these limitations, a key strength of this review is the integrated analysis of rosemary extract together with its major bioactive constituents, allowing a clearer understanding of both shared and compound-specific mechanisms involved in prostate cancer. Moreover, this synthesis addresses the growing yet highly fragmented body of literature on rosemary-derived compounds, providing a comprehensive overview that may help clarify mechanistic patterns and guide future experimental and translational research.

Conclusion

Rosemary and its main components, including betulinic acid, carnosic acid, carnosol, rosmarinic acid, and ursolic acid, have attracted growing scientific interest for their potential role in prostate cancer prevention and therapy. The evidence reviewed indicates that these compounds can influence multiple biological processes involved in tumor development and progression, including cell proliferation, apoptosis, oxidative stress, inflammation, androgen receptor signaling, and metabolic regulation. Many of these effects converge on key oncogenic pathways, including PI3K/Akt/mTOR, NF-κB, STAT3, and AMPK, highlighting the capacity of rosemary-derived phytochemicals to modulate interconnected signaling networks relevant to prostate cancer biology.

At the same time, individual compounds exhibit distinct molecular activities that may complement one another, suggesting that rosemary phytochemicals act as multi-target modulators rather than single-pathway inhibitors. This multi-mechanistic profile may be particularly relevant in prostate cancer, where redundancy among signaling pathways and extensive cross-talk frequently contribute to disease progression and the development of therapeutic resistance.

Despite encouraging preclinical findings, the current body of evidence remains largely based on *in vitro* and animal studies, and the absence of well-designed clinical trials in patients with prostate cancer represents a major limitation of the current literature. Importantly, most mechanistic evidence has been generated using laboratory-based concentrations or experimental animal doses that cannot be directly extrapolated to human cancer therapy. Therefore, based on the currently available evidence, there is no validated or clinically viable pathway for applying rosemary-derived phytochemicals as therapeutic agents in prostate cancer treatment. Future research should therefore focus on improving the pharmacokinetic characterization of these compounds, optimizing delivery strategies, and conducting well-designed *in vivo* and clinical investigations to better determine their safety, bioavailability, and potential therapeutic relevance.

Briefly, rosemary-derived phytochemicals are a promising area of investigation in prostate cancer research; however, their current role should be considered experimental and hypothesis-generating rather than clinically established. Until robust human clinical trials, pharmacokinetic studies, and dose-translation analyses are available, these compounds cannot be recommended for prostate cancer treatment or as substitutes for standard oncological therapies. Continued efforts to clarify their molecular mechanisms and translational potential may contribute to the development of novel complementary strategies for prostate cancer prevention and management.

Acknowledgment

This work was supported by the Clinical Research Development Unit, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.

Funding

The authors declare that no funding, grants, or other support was received during the preparation of this manuscript.

Data Availability of Data and Materials

No new data were created or analyzed during this study. Data sharing does not apply to this article.

Authors' Contributions

K N, AV M, H S, SA E, and BK wrote the manuscript. J J rose to the occasion and helped to gather the data. M GR supervised. All authors approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

During the preparation of this work, the author(s) used ChatGPT and QuillBot to rephrase and reduce plagiarism, improve language and grammar, and finalize the images. After using these tools/services, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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