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Review Article

Rosemary and Melanoma: A Narrative Review of Bioactive Constituents and Therapeutic Pathways

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Abstract

Introduction: Melanoma is an aggressive form of skin cancer characterized by rapid progression, metastasis, and resistance to conventional treatments. Despite advancements in targeted therapies, the need for novel, effective, and safer therapeutic strategies remains critical. Rosemary (*Rosmarinus officinalis* L.; syn. *Salvia rosmarinus* Spenn.), a medicinal herb with potent bioactive compounds—including betulinic acid, carnosic acid, carnosol, rosmarinic acid, and ursolic acid—exhibits anti-inflammatory, antioxidant, and anticancer properties. This review aims to systematically assess the potential of rosemary and its constituents in melanoma prevention and treatment.

Methods: A comprehensive literature review of all relevant *in vivo* and *in vitro* studies was conducted up to May 2026 using Google Scholar, PubMed, Scopus, and Web of Science databases. Studies examining rosemary and its main components, as well as their effects on melanoma cell lines and animal models, were analyzed.

Results: Rosemary and its main components exert multidimensional anti-melanoma effects, modulating oncogenic signaling pathways, inducing apoptosis, suppressing proliferation, and disrupting focal adhesion to impair tumor invasiveness. They also enhance oxidative stress responses, influencing redox balance and ferroptosis induction in melanoma cells. Advances in nanotechnology-driven delivery systems have further optimized bioavailability and therapeutic efficacy, reinforcing the synergistic potential of rosemary extracts when combined with conventional therapies such as chemotherapy and radiotherapy.

Conclusion: Rosemary and its bioactive compounds show strong potential in melanoma therapy, targeting metabolism, oxidative stress, and apoptosis while enhancing conventional treatments. However, clinical validation remains limited. Future research should focus on optimized formulations and combination therapies to establish their role in melanoma management.

Introduction

The skin, the body's largest organ, is continuously exposed to environmental challenges, including microbial infections, physical damage, and ultraviolet (UV) radiation. In 2024, melanoma was reported to be the 17th most common cancer worldwide, ranking 22nd in cancer-related mortality.[1] According to the Global Cancer Observatory (GLOBOCAN), approximately 20 million new cancer cases and 9.7 million cancer-related deaths were reported worldwide in 2022, highlighting the growing global cancer burden. Furthermore, projections suggest that the number of cancer cases may increase to 35 million by 2050 due to demographic changes. Notably, recent trends indicate a gradual increase in melanoma incidence across Asia, underscoring the importance of further research into its regional prevalence and therapeutic approaches.[1,2] Melanoma occurs more frequently in women than in men under the age of 50. However, its incidence in men increases significantly with age, becoming twice as common of 65 years and three times more prevalent by 80 years.[3]

The mortality rate among Asian individuals diagnosed with melanoma is 27% higher compared to their Caucasian equivalents.[4] Superficial spreading melanoma accounts for approximately 70% of all melanoma cases, making it the most frequently recognized subtype. Melanoma develops due to the malignant transformation of melanocytes, the specialized cells responsible for melanin synthesis, which determines skin pigmentation. While it predominantly appears on areas exposed to sunlight, it can also manifest in less visible locations such as the throat, eyes, and nasal passages. Although its exact etiology remains uncertain, prolonged exposure to UV radiation—whether from sunlight or artificial sources like tanning beds—is considered a major risk factor. Individuals experiencing extensive UV exposure are more susceptible to developing melanoma and may also exhibit alterations in skin

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pigmentation.[1] Melanoma primarily affects individuals aged in their mid-40s and older and is characterized by its aggressive metastatic nature.[5] Due to its ability to progress silently without noticeable symptoms, timely detection is essential for successful management.[6] Early diagnosis significantly enhances treatment outcomes, improving patient prognosis.[7]

Standard melanoma treatments include chemotherapy, immunotherapy, radiation therapy, surgical excision, and targeted therapies. However, the inherent biological complexities of melanoma, including its adaptability and resistance mechanisms, limit the effectiveness of these interventions. Additionally, these treatments are often associated with considerable side effects and financial burdens.[8] Considering these challenges, researchers have increasingly explored alternative and complementary approaches to melanoma management. Among these, herbal medicines have attracted significant scientific interest for decades.[9-11]

Rosemary (*Rosmarinus officinalis* L.; syn. *Salvia rosmarinus* Spenn.), originally native to the Mediterranean, has a remarkable ability to thrive in diverse climates, allowing it to be cultivated globally. This aromatic, evergreen shrub is distinguished by its dense foliage, which contributes to its distinct fragrance. Beyond its medicinal applications, rosemary is commonly incorporated into culinary practices as a seasoning, preservative, and flavor enhancer.[12] Phytochemical investigations have identified a range of bioactive polyphenols present in rosemary extract. These include triterpenoids like betulinic acid and ursolic acid[13], phenolic acids such as rosmarinic acid, caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, and quinic acid, along with phenolic diterpenes like carnosic acid, carnosol, epirosmanol, isorosmanol, and rosmanol. Additionally, rosemary contains flavonoids such as genkwanin, kaempferol, quercetin, and rutin (Figure 1) all of which contribute to its therapeutic properties.[14]

Studies have highlighted the wide-ranging biological properties of rosemary and its main components, revealing their potential in various therapeutic applications, including neuroprotective,[15] anti-sarcopenic,[16] antidiabetic, antihypertensive, anti-dyslipidemic,[17] nephroprotective,[18] anti-dyspnea,[19] antinociceptive,[20] anti-apoptotic,[21] antidote,[22] cardioprotective,[23,24] antioxidant,[25] antirheumatic,[26] anti-inflammatory,[27] hypnotic,[28] antidepressant,[29] anti-obesity,[30] hepatoprotective,[31] and anti-tumor activities.[32,33] Rosemary is generally considered safe for food preservation; however, it should be used in moderation. Excessive intake may pose risks, including possible teratogenic effects, as well as harm to the reproductive system, liver, and kidneys. Furthermore, it is crucial to be aware of its potential interactions with various medications.[34]

This narrative review aims to systematically explore and synthesize existing *in vivo* and *in vitro* studies evaluating the therapeutic mechanisms of rosemary and its major constituents in melanoma management. By bringing together current scientific findings, it aims to clarify how rosemary-derived compounds interact at the molecular level, their medicinal properties, and possible applications in clinical fields. Eventually, this review may facilitate the development of new plant-based approaches to prevent and treat melanoma, supporting further laboratory and clinical research.

Methods

Search strategy

For this narrative review, a literature search was conducted to evaluate the therapeutic mechanisms of rosemary (*Rosmarinus officinalis* L.; syn. *Salvia rosmarinus* Spenn.) and its main components in melanoma. Relevant studies published up to May 2026 were retrieved from the following databases: Google Scholar, PubMed, Scopus, and Web of Science. The search was intended to identify relevant publications and provide a broad overview of existing evidence rather than to perform a formal systematic review. To ensure a thorough literature review, the following keywords were used: "Rosemary", "*Rosmarinus officinalis*", "*Salvia rosmarinus*", "Betulinic acid", "Carnosic acid", "Carnosol", "Rosmarinic acid", "Ursolic acid", "Melanoma", "*In vivo*", "*In vitro*" and "Clinical trials".

Inclusion criteria

Studies were considered for inclusion based on their relevance to the topic of rosemary and melanoma.

- Studies published in peer-reviewed journals up to May 2026.
- *In vivo* and *in vitro* studies evaluating rosemary or its main components in melanoma models.
- Research investigating molecular mechanisms, therapeutic efficacy, or combination approaches with existing treatments.
- Articles available in English.

Exclusion criteria

Studies were excluded when they were not relevant to the scope of this narrative review, including:

- Studies unrelated to rosemary or melanoma.
- Research lacking experimental data (e.g., commentary articles, opinions, or unrelated reviews).
- Papers focusing on non-melanoma cancers or general antioxidant effects without relevance to melanoma.
- Articles with insufficient methodological details or data reliability concerns.

Data extraction and analysis

Relevant information was summarized and narratively synthesized from selected studies, including:

- Study type: *In vivo* or *in vitro*
- Melanoma models: Cell lines or animal studies
- Intervention: Rosemary extract or individual compounds (betulinic acid, carnosic acid, carnosol, rosmarinic acid, ursolic acid)
- Mechanisms: Effects on apoptosis, oxidative stress, inflammation, and tumor progression
- Outcomes: Anticancer efficacy, toxicity profile,

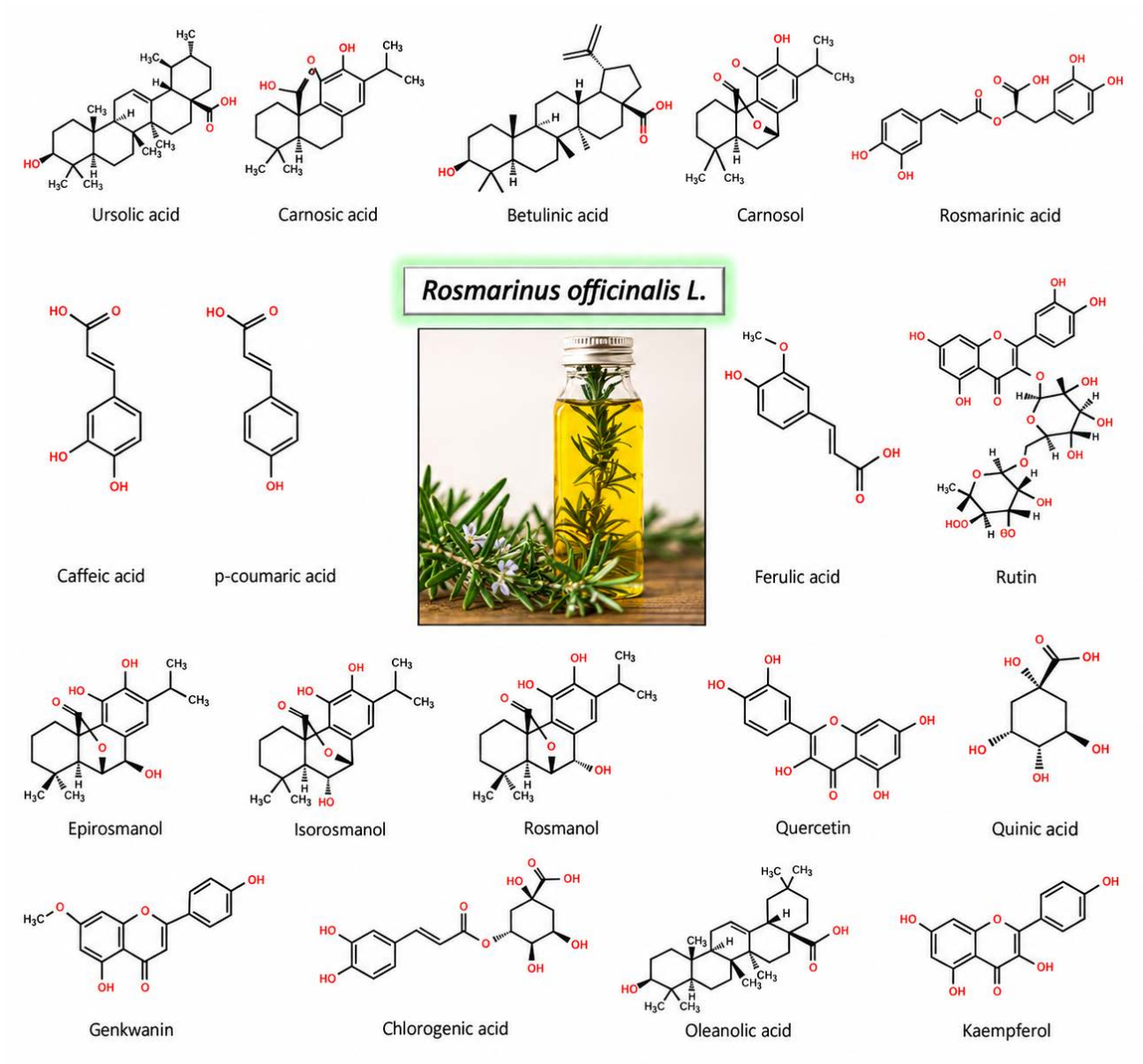


Figure 1. Chemical structures of key bioactive compounds found in rosemary (Images from https://www.freepik.com)

and potential synergistic effects with conventional therapies

The collected information was narratively integrated to highlight key findings, therapeutic mechanisms, and emerging research directions related to the role of rosemary in melanoma treatment.

Effect of rosemary and its main components on melanoma

Rosemary extracts or oil

In vitro

Assessing the therapeutic potential of rosemary leaves methanolic extract on M14 and A375 human melanoma cell lines indicated its ability to protect against nitric oxide (NO)-induced cleavage of plasmid Bolivar and Rodriguez 322 (pBR322) deoxyribonucleic acid (DNA). Notably, the extract significantly inhibited the proliferation of M14 and A375 human melanoma cell lines. Moreover, findings suggest that the extract induces apoptotic cell death in these melanoma cells. Importantly, no significant elevation in lactate dehydrogenase (LDH) release was detected, indicating that genomic DNA fragmentation, confirmed via COMET (single-cell gel electrophoresis) assay, was associated with apoptosis rather than necrosis.[35]

An *in vitro* study demonstrated that a hydro-alcoholic extract of rosemary effectively inhibited the proliferation of human melanoma A375 cells in a dose- and time-dependent manner. Cell cycle analysis revealed that the extract exerted both cytostatic and cytotoxic effects to suppress tumor growth, while assessments of reactive oxygen species (ROS) levels and protein carbonylation indicated that its antiproliferative action did not arise from a pro-oxidant mechanism. Chemical profiling allowed for the identification of key constituents—luteolin, carnosol, scutellarin, rosmarinic acid, and apigenin—whose individual cytotoxic activities were evaluated over 24-72 hours. Among these, apigenin, luteolin, and carnosol displayed significantly higher efficacy than scutellarin and

rosmarinic acid. Further analysis showed that treatments at lower (1:120 and 1:240) dilutions significantly impacted cell cycle progression. The 1:120 dilution led to an increase of up to 30% of cells in the sub-G0 phase, indicative of apoptosis, alongside a marked reduction in the G0/G1 phase population. Conversely, the 1:240 dilution induced a substantial decrease in G0/G1 phase cells, accompanied by cell cycle arrest in the G2/M phase and the emergence of hyperploid cells. Proteomic investigation further revealed that rosemary treatment downregulated proteins essential for maintaining cellular homeostasis, likely inducing endoplasmic reticulum stress, thereby disrupting critical cellular functions.[36]

Topical 5-fluorouracil (5-FU) remains a widely used treatment for skin cancer, yet its effectiveness is often slowed down by drug resistance and adverse effects. Therefore, a study explored the potential of rosemary extract as a complementary agent due to its dose-dependent antitumor activity and ability to enhance 5-FU sensitivity in resistant cells. To achieve controlled drug release, polymeric nanofibers encapsulating both 5-FU and rosemary extract were optimized using electrospinning. Cytotoxicity assays demonstrated significantly enhanced anticancer activity compared to control groups.[37]

The potential effect of rosemary essential oil and its major constituent, α-pinene, in melanoma treatment was assessed, particularly when formulated using nanotechnology. Alginate nanoparticles were successfully formulated with α-pinene and rosemary essential oil, with encapsulation. Evaluation of nanoparticle cytotoxicity demonstrated that Alginate-free formulations had no significant effect on A375 cell viability compared to untreated controls. In contrast, both Alginate-pinene and Alginate-rosemary exhibited dose-dependent cytotoxic effects. At the highest concentration, Alginate-pinene induced a significantly greater reduction in cell viability than Alginate-rosemary, though no substantial difference was observed at lower doses. Despite Alginate-pinene demonstrating slightly greater potency, the difference between the two treatments was not statistically significant.[38]

Chitosan nanoparticles encapsulating rosemary essential oil and α-pinene were synthesized and their effects were examined on A375 cells. Notably, α-pinene-loaded chitosan nanoparticles exhibited superior anticancer efficacy compared to nanoparticles containing rosemary essential oil, non-formulated essential oil, and non-formulated α-pinene. Apoptotic activity was confirmed in all samples, as indicated by a Bcl-2-associated X (Bax)/B-cell lymphoma protein 2 (Bcl-2) ratio greater than one.[39]

In B16-F10 cells, rosemary essential oil was found to influence cell cycle progression and cell death mechanisms. Quantitative proteomics revealed 165 differentially expressed proteins, with several linked to ferroptosis. Rosemary essential oil treatment led to increased ROS generation, mitochondrial membrane potential disruption, and lipid peroxidation. Further experiments confirmed substantial modifications in ferroptosis-related gene expression. Remarkably, the antiproliferative and ROS-enhancing effects of rosemary essential oil were prevented by ferroptosis inhibitors[40] (Table 1).

Furthermore, another study demonstrated the potential of nano-emulsified rosemary essential oil as a promising therapeutic agent for melanoma treatment. Following synthesis and characterization, the nano-emulsion exhibited significant cytotoxic effects on A375 melanoma cells, effectively reducing cell viability and inhibiting angiogenesis. Moreover, treatment with this formulation enhanced apoptotic activity, as evidenced by increased expression of the pro-apoptotic marker caspase-3.[41]

The ethanolic extract of rosemary significantly inhibited the proliferation of metastatic melanoma cell lines, with effective concentrations below 0.1 mg/ml for A375 cells and below 0.05 mg/ml for SK-MEL-28 cells after 24 hours of treatment. Additionally, wound-healing assays

Table 1. Effects of rosemary on melanoma

Compound	Study design	Doses/Duration	Results	Ref.
In vitro				
Rosemary leaves methanolic extract	M14 and A375 cells	10, 20, 40, and 80 µg/ml, 72 h	↑ DNA damage, percentage of the fragmented DNA, TMOM ↓ Cell growth	35
Rosemary leaves hydro-alcoholic extract	A375 cells and B16F10 murine melanoma cells	20 µM, 24-72 h	↑ Cytotoxic and cytostatic effects, apoptosis, endoplasmic reticulum stress ↓ Cell growth, ells proliferation, proteins essential for maintaining cellular homeostasis, cellular functions	36
Polymeric nanofibers loaded with 5-FU and rosemary	A375 cell	-	↑ Anticancer activity	37
Rosemary essential oil	A375 cells	50, 100, 200, 400, and 800 µg/ml, 24 h	↑ Cytotoxicity, Bax/Bcl-2 ratio	38
Chitosan nanoparticles encapsulating rosemary essential oil and α-pinene	A375 cells	-	↑ Anticancer efficacy, apoptotic activity, Bax/Bcl-2	39
Rosemary essential oil	B16F10 cells	-	↑ ROS generation, mitochondrial membrane potential disruption, lipid peroxidation, ferroptosis	40
Nano-emulsified rosemary essential oil	A375 cells	30 and 300 µg/ml	↑ Apoptotic activity, expression of caspase-3 ↓ Cell viability, angiogenesis	41
Rosemary ethanolic extract	A375 and SK-MEL-28 cells	0.005-0.2 mg/ml	↓ Proliferation, cell migration	42

Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; DNA: deoxyribonucleic acid; ROS: reactive oxygen species; TMOM: tail moment.

demonstrated a significant reduction in cell migration, indicating an antimigratory effect.[42]

In general, rosemary exhibits significant promise as a therapeutic agent against melanoma, primarily due to the synergistic interactions among its diverse bioactive compounds. Rather than relying on isolated components, the collective effect of polyphenols, flavonoids, and terpenes contributes to its antiproliferative, apoptotic, and anti-angiogenic properties. Rosemary extract and essential oil have been shown to induce apoptosis through caspase activation, disrupt melanoma cell cycle progression, and inhibit angiogenesis—collectively limiting tumor growth and survival. Furthermore, rosemary-based formulations, particularly those utilizing nanotechnology, enhance drug delivery efficacy and potentially overcome conventional treatment barriers.

Despite its strengths, including its complex mechanisms and potential for combination with existing therapies, current research is limited to *in vitro* investigations. No *in vivo* or clinical trials have validated its efficacy or safety in human melanoma treatment. Additionally, while studies highlight apoptosis without inducing necrosis, further exploration is needed to ensure selective cytotoxicity without compromising healthy tissue. To translate the therapeutic potential of rosemary into clinical application, future studies should explore optimized formulations, bioavailability improvements, and comprehensive animal model assessments. The integration of rosemary-derived compounds into melanoma treatment strategies could offer an innovative, natural, and targeted method, establishing a foundation for novel cancer management solutions.

Betulinic acid

In vitro

Betulinic acid has been shown to suppress the enzymatic activity of aminopeptidase N (EC 3.4.11.2) in a concentration-dependent manner. The study found that betulinic acid had a lower half-maximal inhibitory concentration (IC_{50}) compared to bestatin, a recognized inhibitor of this enzyme, indicating its stronger inhibitory potential.[43]

The evaluation of the selective apoptotic effects of betulinic acid in melanoma disclosed that, in human metastatic C8161 melanoma cells, treatment with betulinic acid led to increased DNA fragmentation, pronounced growth arrest, and accelerated loss of viability, whereas non-metastatic C8161/neo 6.3 cells exhibited a weaker response. Besides, betulinic acid was found to induce p53 expression without triggering p21WAF1 activation, a pattern observed in metastatic Mel Juso cells but not in their non-metastatic Mel Juso/neo 6 counterparts. In addition, bromodeoxyuridine enhanced the apoptotic effects of betulinic acid in metastatic Mel Juso cells.[44]

Modifications of betulinic acid through coupling with various amino acids at the C-28 carboxylic acid position have been explored to assess their impact on cytotoxicity against human melanoma (MEL-2) and human epidermoid carcinoma (KB) cell lines. Several of these conjugates exhibited enhanced water solubility and selective cytotoxicity.[45]

Another *in vitro* study investigated the cytotoxicity of betulinic acid and its metabolites 3-oxo-lup-20(29)-en-28-oic acid, 3-oxo-11α-hydroxy-lup-20(29)-en-28-oic acid, 1β-hydroxy-3-oxo-lup-20(29)-en-28-oic acid, and 3β,7β,15α-trihydroxy-lup-20(29)-en-28-oic acid (2 to 5) against human melanoma cell lines Mel-1 (lymph node) and Mel-2 (pleural fluid). The findings revealed differential activity among the metabolites, with metabolites 3 to 5 showing no cytotoxic effect against Mel-1 and metabolite 5 also lacking activity against Mel-2. In contrast, metabolites 2 to 4 demonstrated enhanced cytotoxicity against Mel-2 compared to the parent compound, while metabolite 2 was less effective than betulinic acid against Mel-1.[46]

Investigations into betulinic acid efficacy, both as a standalone treatment and in combination with ionizing radiation, revealed a consistent suppression of cell proliferation and colony formation across various melanoma cell lines. The combination of betulinic acid with ionizing radiation exhibited an additive effect on growth inhibition in colony-forming assays. Apoptotic induction was also confirmed. Moreover, betulinic acid exerted a stronger growth-inhibitory effect on melanoma cells compared to normal human melanocytes. Despite promoting apoptosis, treatment with betulinic acid led to increased expression of the anti-apoptotic protein Mcl-1. Furthermore, its antiproliferative activity appeared to be independent of p53 status.[47]

Likewise, another study explored the regulation and functional relevance of Mcl-1 in human melanoma cells, highlighting its induction by betulinic acid. Using Mcl-1 phosphorothioate antisense oligonucleotides, researchers examined the effects of downregulating Mcl-1 expression, while phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) inhibitors helped elucidate its regulatory pathways. The findings indicated that betulinic acid upregulates Mcl-1 via a signal-transduction mechanism inhibited by LY294002 and wortmannin, affecting protein kinase B (Akt) phosphorylation. Furthermore, PD98059 reduced Mcl-1 levels, an effect reversed by Akt. Additionally, combining Akt with Mcl-1 antisense oligonucleotides resulted in a synergistic inhibition of melanoma cell growth.[48]

Investigating the apoptotic and cytotoxic effects of betulinic acid in human melanoma cells under varying pH conditions, particularly in combination with acute acidification and hyperthermia, demonstrated that betulinic acid induced higher levels of apoptosis in low pH-adapted DB-1 melanoma cells compared to those grown at physiological pH (7.3). Acute acidification further sensitized these cells to betulinic acid-mediated apoptosis and cytotoxicity. Moreover, betulinic acid enhanced susceptibility to hyperthermia-induced cell death in cells adapted to acidic conditions (pH 6.7) or acutely acidified to pH 6.3, whereas cells maintained at pH 7.3 showed no comparable response. Survival rates of acidified or pH-adapted cells exposed to betulinic acid and hyperthermia

ranged from 2-9%, reinforcing its potential as a synergistic therapeutic strategy.[49]

Treatment of UISO-Mel-1 cells with betulinic acid activated the pro-apoptotic MAPKs, including p38 and stress-activated protein kinase/c-Jun NH(2)-terminal kinase, while leaving extracellular signal-regulated kinases (an anti-apoptotic MAPK) unaffected. The findings also suggested a critical link between MAPKs and ROS with antioxidant pre-incubation preventing both apoptosis and MAPK phosphorylation. Additionally, betulinic acid disrupted mitochondrial membrane potential, though caspase involvement was ruled out.[50]

Treating murine melanoma B16 cells with betulinic acid and its hydroxylated derivatives led to a dose- and time-dependent increase in ROS production and mitochondrial membrane potential dissipation, ultimately triggering apoptosis. Cell cycle analysis revealed that 3-oxo-23-hydroxybetulinic acid and 23-hydroxybetulinic acid significantly enhanced DNA fragmentation while reducing the G1 cell population.[51]

A study evaluated the combined antitumor effects of betulinic acid and vincristine on B16F10 murine melanoma cells. Betulinic acid exhibited a synergistic cytotoxic effect when used alongside vincristine, with each compound inducing cell cycle arrest at distinct phases— betulinic acid at G1 and vincristine at G2/M—leading to apoptosis in melanoma cells.[52]

An *in vitro* study was carried out to explore the mechanisms underlying melanoma cell resistance to betulinic acid. The results revealed that human melanoma cells exhibited reduced sensitivity to betulinic acid compared to keratinocytes, likely due to transient activation of the epithelial growth factor receptor (EGFR)/Akt pathway and survivin expression. Specifically, betulinic acid treatment induced survivin expression within 30 minutes, peaking at 2 hours and persisting for 8 hours before returning to baseline at 24 hours. Similarly, epithelial growth factor (EGF) stimulation led to survivin upregulation. Further analysis showed betulinic acid triggered EGFR tyrosine phosphorylation and robust Akt activation, which peaked at 1 hour and remained elevated for 4 hours. Moreover, betulinic acid stimulated extracellular signal-regulated kinase (ERK) activation but had a weaker effect on p38 and c-Jun N-terminal kinase (JNK) pathways. Notably, pretreatment with the EGFR inhibitor PD153035 suppressed betulinic acid-induced EGFR phosphorylation, ERK and Akt activation, and survivin expression, enhancing melanoma cell death when combined with betulinic acid.[53]

Examining the role of U0126, a MAPK/ERK kinase (MEK)1/2 inhibitor, in modulating melanoma responses to UV radiation and betulinic acid demonstrated that U0126 suppresses early response ERK activation and cyclin A expression in wt p53 C8161 melanoma cells exposed to either stimulus. While U0126 does not mitigate UV-induced damage, it effectively counteracts betulinic acid-mediated apoptosis across multiple melanoma cell lines, including mutant p53 WM164 and wt p53 MelJuso. Notably, WM164 melanoma cells displayed significant cyclin-dependent kinase 4 (CDK4) protein reduction, a unique apoptotic marker absent in other key cell cycle regulators. Furthermore, betulinic acid induced chromatin condensation in both adherent and spheroid WM164 cells, an effect reversed by U0126.[54]

Investigating the cytotoxic effects of betulinic acid on normal melanocyte cell lines and both drug-resistant and drug-sensitive melanoma cell lines revealed pronounced cytotoxic activity in all cell lines; however, the strongest effect was observed in NHEM-neo cells. Apoptotic cell death was further validated via flow cytometry and caspase-3 immunocytochemical staining.[55]

Exploring the role of betulinic acid in regulating epithelial-to-mesenchymal transition, a critical process in cancer metastasis influenced by neutrophil gelatinase-associated lipocalin (NGAL) indicated that betulinic acid effectively suppresses epithelial-to-mesenchymal transition in A375 melanoma cells by downregulating mesenchymal markers while enhancing epithelial characteristics, thereby preventing cytoskeletal reorganization. In addition, betulinic acid reduces endogenous NGAL levels and inhibits epithelial-to-mesenchymal transition triggered by exogenously added NGAL, leading to a diminished invasive cellular phenotype.[56]

The cytotoxic effects of picolyl amides of betulinic acid (3a-3c and 6a-6c) and their structure-activity relationships based on pyridine ring substitution were assessed. Among the tested compounds, 3a and 3b exhibited notable cytotoxicity against the G-361 melanoma cell line. Their therapeutic selectivity was significant, with a therapeutic index of 100 for G-361 melanoma cells versus normal fibroblasts. In contrast, other amides (3c and 6a-6c) displayed weaker cytotoxic effects. Compounds 3a-3c demonstrated significant apoptotic and antiproliferative effects in G-361 cells. Moreover, flow cytometry revealed an elevated presence of apoptotic cells in the sub-G1 phase of the cell cycle within the G-361 cell line following exposure to compounds 3a and 3b. Conversely, treatment with 3c did not lead to an increase in sub-G1 cells and had no impact on the overall cell cycle distribution. Western blot analysis confirmed caspase-7 degradation and poly (ADP-ribose) polymerase (PARP) cleavage, highlighting the involvement of caspase-mediated apoptosis. Additionally, 3a and 3b downregulated phospho-Erk1/2 (Thr202/Tyr204), disrupting pathways related to proliferation and differentiation. Caspase-3/7 activity assays further validated apoptosis induction, with 3a increasing activity up to eighteen-fold and 3b inducing a twelve-fold increase. In contrast, 3c did not activate caspases or alter signaling pathways.[57]

Another study highlighted the biomedical potential of magnetoliposomes loaded with betulinic acid (BA-Fe_3O_4@Lip), due to their preserved magnetic properties and cytotoxic effects. The findings provide preliminary evidence of their impact on melanoma cell lines A375 and B164A5, demonstrating significant cytotoxicity compared to blank liposomal structures. In contrast, the healthy

epidermal JB6 Cl 41-5a cell line maintained a high viability rate following exposure to BA-Fe_3O_4@Lip[58] (Table 2).

Betulinic acid was examined for its anticancer effects on primary equine melanoma cells and dermal fibroblasts, as well as its ability to permeate isolated equine skin. Results indicate that betulinic acid exerts significant antiproliferative and cytotoxic effects in a dose- and time-dependent manner. Furthermore, betulinic acid successfully penetrated the required skin layers *in vitro*, supporting its feasibility as a topical agent.[66]

In A375 human melanoma cells, betulinic acid exhibited a dose-dependent inhibitory effect on both mitochondrial glycolysis and respiration. At sub-toxic concentrations, it induced mitochondrial dysfunction, characterized by alterations in localization and morphology and a decline in mitochondrial membrane potential. Furthermore, betulinic acid triggered apoptotic features, including nuclear fragmentation, apoptotic body formation, and upregulation of pro-apoptotic markers (Bax, Bcl-2-associated death promoter (Bad), and Bcl-2 homologous

Table 2. Effects of betulinic acid on melanoma

Compound	Study design	Doses/Duration	Results	Ref.
In vitro				
Betulinic acid	Mel-1,2,3,4 cells	1-5 µg/ml, 0-72 h	↓ Cell growth	59
Betulinic acid	C8161, C8161/neo 6.3, Mel Juso, and Mel Juso/neo 6 cells	1, 1.5, and 2 µg/ml, 0-96 h	↑ DNA fragmentation, cell viability, p53 expression ↓ Cell growth	44
Betulinic acid	Mel-1 and Mel-2 cells	-	↑ Anti-melanoma potential	46
Betulinic acid	Normal human epidermal melanocytes, A375, 518A2, MES20, MES21, Neo II-tr, Neo-IV-tr	0-10 µg/ml	↑ Growth inhibition, apoptotic induction, expression of Mcl-1 ↓ Cell proliferation, colony formation	47
Betulinic acid	Me665/2/21 and Me665/2/6 cells	0.01-10 µg/ml	↑ Antiproliferative	60
Betulinic acid	518A2, 518-L1 (61-leu/L), 518-neo, M20, NMel-II cells	10 µg/ml	↑ Mcl-1	48
Betulinic acid	DB-1 cells	4 and 8 µg/ml, 24 and 48 h	↑ Apoptosis in low pH-adapted melanoma cells, susceptibility to hyperthermia-induced cell death in cells adapted to acidic conditions	49
Betulinic acid	UISO-Mel-1 cells	0 –32 µg/ml	↑ p38, stress-activated protein kinase/ JNK, disruption of mitochondrial membrane potential	50
Betulinic acid	B16 cells	0-1000 µg/ml, 1-72 h	↑ Mitochondrial membrane dissipation, ROS production, apoptosis, DNA fragmentation ↓G1 cell population	51
Betulinic acid	B16F10 cells	1, 2.5, and 5 µM	↑ Cell cycle arrest, apoptosis	52
Betulinic acid	IGR1 cells	0-20 µg/ml, 15, 24 and 48 h	↑ Apoptosis, DNA fragmentation, morphological alterations	61
20,29-Dihydro-20,29-dichloromethylenebetulinic acid	Colo 38 and Bro lines	10 mM	↑ Cytotoxicity ↓ Cell growth	62
Betulinic acid	C8161 and Mel Juso cells	3.5 and 5 µg/ml, 4–6 days	↑ Apoptosis, chromatin condensation, ERK signaling	54
Betulinic acid and its four derivatives (2–5)	A375 cells	-	Derivative 5: ↓ IC_{50} value	63
Betulinic acid	A375 cells	-	↑ Epithelial characteristics ↓ Epithelial-to-mesenchymal transition, cytoskeletal reorganization, endogenous NGAL levels, invasive cellular phenotype	56
Betulinic acid derivative (octakis-[6-deoxy-6-(2-sulfanyl ethanesulfonic acid)]-γ-cyclodextrin)	B164A5 cells	10 mM	↑ Antiproliferative activity	64
Betulinic acid and its derivatives (B10 and NVX-207)	MelDuWi and MellJess/HoMelZh, A375 cells	0-100 µM, 2-96 h	↑ Cytotoxicity, DNA fragmentation, apoptosis, caspase activation, phosphatidylserine externalization	65
Picolyl amides of betulinic acid (3a-3c and 6a-6c)	G-361 cells	0.5-25 µM, 24 h	3a and 3b: - Therapeutic index100 for G-361 cells versus normal fibroblasts ↑ Cytotoxicity, apoptosis	57
Magnetoliposomes loaded with betulinic acid	A375, B164A5, and JB6 Cl 41-5a cells	5 and 25µM, 24 and 48 h	↑ Cytotoxicity	58
Betulinic acid	eRGO1 and MelDuW cells	1 to 100 µmol/l, 24-96 h	↑ Antiproliferative and cytotoxic effects	66
Betulinic acid	A375 cells	1, 5, 10, 20, 25 and 50 µM, 24 h	↑ Mitochondrial dysfunction, alterations in mitochondrial morphology and localization, apoptotic body formation, nuclear fragmentation, Bad, Bak, Bax ↓ Mitochondrial glycolysis and respiration, mitochondrial membrane potential	67

Compound	Study design	Doses/Duration	Results	Ref.
In vitro				
Betulinic acid	FM55P, FM55M2, A375, SK-MEL 28	1-40 μM, 72 h	- Selectively targeted melanoma cells while sparing normal keratinocytes	68
Betulinic acid	SK-Mel-28 and RPMI-7951	1, 5, 10, and 25 μM, 24 h	↑ Cytotoxic activity, actin fiber alterations, apoptotic bodies, cell detachment, cell rounding, chromatin condensation	69
Betulinic acid	SK-MEL-5 and B16F10 cells	1-75 μM, 24 and 72 h	↑ Cytotoxic activity	70
Betulinic acid-functionalized gold nanoparticles	RPMI-7951 cells	10, 25, and 50 μM, 24 and 48 h	↑ Selective cytotoxic effects, antiproliferative activity, morphological alterations, Bax ↓Bcl-2, mitochondrial respiration	71
C30-1,2,4-triazole derivative of betulinic acid	RPMI-7951 cells	0.08, 0.4, 2, 10 and 50 μM, 24 h	↑ Cytotoxicity, apoptosis induction at the mitochondrial level ↓ Cell viability	72
Betulinic acid Derivatives (BA1, BA2)	A375 cells	1, 10, 25, 50, and 75 μM, 72 h	↑ Antiproliferative effects	73
Betulinic acid Derivatives (BA1, BA2)	B164A5 cells	1, 10, 25, 50, and 75 μM, 72 h	↑ Cytotoxicity, membrane damage, lysosome disruption, apoptosis ↓ Cell migration	74
Betulinic acid fatty esters and their corresponding liposomal formulations	A375 cells	10-100 μM, 24 and 48 h	↑ Cytotoxic activity, apoptotic changes, Bax expression ↓ Cell migration, Bcl-2	75
In vivo				
Betulinic acid	Female C57BL/6 mice	10 mg/kg, 2 five-day, i.p.	↓ Tumor cell metastasis to the lung, lung nodules larger than 1 mm	52
Betulinic acid	C57BL/6J mice	Topical: Twice a day, 20 days Intraperitoneal: 0.5 ml, 10 days, i.p.	Topical: ↑ Recovery of the skin ↓ Erythema and intense pigmentation Intraperitoneal: ↓ Metastatic process to the liver	76
Betulinic acid	Female C57BL/6 mice	20 mg/kg, 18 days, i.p.	↑Respiratory function in liver mitochondria in basal and active respiration	77
Betulinic acid derivative (cyclodextrin derivative—octakis-[6-deoxy-6-(2-sulfanyl ethanesulfonic acid)]-γ-cyclodextrin)	Female C57BL/6J mice	100 mg/kg, 21 days, i.p.	↑ Skin integrity ↓ Tumor volume and weight, melanin and erythema levels, transepidermal water loss values	64
Betulinic acid and its derivative NVX-207	*Lipizzaner mares*	1%, twice daily, 91 days	↓ Tumor volume	78

Bad: Bcl-2-associated death promoter; Bak: Bcl-2 homologous antagonist/killer; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma protein 2; DNA: deoxyribonucleic acid; ERK: extracellular signal-regulated kinase; IC_{50}: half-maximal inhibitory concentration; JNK: c-Jun N-terminal kinase; NGAL: neutrophil gelatinase-associated lipocalin; ROS: reactive oxygen species.

antagonist/killer (Bak)).[67]

The findings of another investigation demonstrated the cytotoxic and antiproliferative effects of betulinic acid, both as a standalone treatment and in combination with taxanes (docetaxel, paclitaxel), across four melanoma cell lines. Isobolographic analysis revealed favorable drug interactions, with betulinic acid co-treatment exhibiting additive effects and a tendency toward synergy. Importantly, betulinic acid selectively targeted melanoma cells while sparing normal keratinocytes (HaCaT), as evidenced by lower LDH release.[68]

Betulinic acid was investigated for its selective apoptotic effects on two human melanoma cell lines (RPMI-7951 and SK-Mel-28). Betulinic acid demonstrated concentration-dependent cytotoxic activity. Morphological analysis revealed apoptotic-like changes, including the formation of apoptotic bodies, chromatin condensation, cell detachment, and cell rounding. Actin fiber alterations further supported apoptotic processes.[69]

This study examines the differential cytotoxic effects of betulinic acid on malignant melanoma cell lines (pigmented and non-pigmented). The findings reveal that betulinic acid exerted a dose-dependent cytotoxic effect on both melanoma and healthy fibroblast cells. However, SK-MEL-5 cells (amelanotic) demonstrated higher sensitivity to betulinic acid compared to B16F10 cells (melanotic).[70]

In vitro assessments revealed that betulinic acid- gold nanoparticles exhibited selective cytotoxic effects and greater antiproliferative activity against RPMI-7951 melanoma cells compared to free betulinic acid. Also, the nano-formulation induced pro-apoptotic effects, evidenced by morphological alterations in melanoma cells and the downregulation of anti-apoptotic Bcl-2 alongside the upregulation of pro-apoptotic Bax. Furthermore, betulinic acid- gold nanoparticles significantly inhibited mitochondrial respiration.[71]

Considering the limited bioavailability of betulinic acid, researchers synthesized and evaluated two novel derivatives—N-(2,3-indolo-betulinoyl)diglycylglycine (BA1) and N-(2,3-indolo-betulinoyl)glycylglycine (BA2)—alongside previously reported compounds BA3, BA4, and natural betulinic acid. *In vitro* evaluations using B164A5

demonstrated enhanced cytotoxicity with the introduction of an indole framework at C2 and amino acid conjugation at C28, with BA2 and BA3 exhibiting approximately 2.20-fold greater inhibitory activity than BA4. Cytotoxicity assessments on HaCaT revealed minimal toxicity at concentrations up to 10 μM, while higher doses significantly reduced viability. Membrane integrity analysis indicated damage in melanoma cells, particularly with BA2 and BA3. Lysosomal disruption appeared dose-dependent, with BA1, BA2, and BA3 showing the strongest cytotoxic effects. Cell migration was inhibited, with BA2 and BA3 being the most effective. Apoptotic markers further differentiated the effects of BA2 from BA3-induced necrosis.[74]

The anticancer potential of three synthesized betulinic acid fatty esters— But-betulinic acid, Pal-betulinic acid, and St-betulinic acid— alongside their corresponding liposomal formulations was tested against A375 and HaCaT cell lines. The findings revealed that both the esters and their liposomal nano-formulations exhibited dose- and time-dependent cytotoxicity against melanoma cells, with But-betulinic acid showing the highest efficacy. Moreover, liposomal formulations—particularly St-betulinic acid-Lip, But- betulinic acid-Lip, and betulinic acid-Lip—demonstrated enhanced cytotoxicity, with lower IC_{50} values compared to non-encapsulated betulinic acid. Morphological assessment indicated apoptotic changes in A375 cells, while HaCaT cell morphology remained unaffected. Additionally, both betulinic acid esters and their liposomal forms suppressed melanoma cell migration. Gene expression analysis further confirmed their pro-apoptotic impact, with increased Bax expression and reduced Bcl-2 levels, particularly in liposomal formulations. Overall, But-betulinic acid and its liposomal formulation exhibited superior anticancer effects.[75]

The effect of betulinic acid and its derivative NVX-207 as therapeutic agents for equine malignant melanoma were explored. The active ingredients, incorporated into either a microemulsion gel or microemulsion, were applied to equine skin samples. The findings showed that all tested formulations surpassed the IC_{50} values necessary for cytotoxic effectiveness against equine melanoma cells, regardless of incubation duration. Notably, NVX-207 administered with oxygen-flow assistance exhibited a significant time-dependent accumulation and depot effect in the skin.[79]

In vivo

Betulinic acid demonstrated therapeutic potential in both topical applications for experimental nevi and systemic treatments for early-stage metastatic melanoma. In topical applications, it promoted skin recovery and reduced intense pigmentation and erythema. In the intraperitoneal model, it attenuated metastatic processes to the liver.[76]

It has also been reported that betulinic acid treatment enhances respiratory function in liver mitochondria isolated from mice with murine melanoma. This improvement is reflected in both basal and active respiration, suggesting a role in mitochondrial bioenergetics that may contribute to its broader therapeutic benefits.[77]

Assessing the effect of betulinic acid: octakis-[6-deoxy-6-(2-sulfanyl ethanesulfonic acid)]-γ-cyclodextrin (GCDG) on mice with melanoma revealed that, tumor volume and weight significantly decreased in treated mice compared to controls, with histological analysis confirming lower melanoma expression in treated samples. Melanin and erythema levels were reduced in the treatment group. Transepidermal water loss values were also lower in treated animals, indicating improved skin integrity.[64]

Another study evaluated the safety and efficacy of topically applied betulinic acid and its derivative NVX-207 in treating early-stage equine melanocytic tumors. *Lipizzaner mares* were divided into three groups, receiving either 1% NVX-207, 1% betulinic acid, or placebo. Results indicated that betulinic acid significantly reduced tumor volume compared to placebo, while NVX-207 showed a trend toward tumor reduction but lacked statistical significance. The treatment was well tolerated and demonstrated safety for topical application.[78]

Generally, betulinic acid presents a compelling therapeutic strategy for melanoma by exerting selective toxicity against malignant cells while sparing normal tissues. Its mechanisms of action encompass apoptotic induction, enzymatic inhibition, mitochondrial dysfunction, and modulation of survival pathways. Betulinic acid disrupts essential proteolytic functions by inhibiting aminopeptidase N activity, while also triggering apoptosis through ROS production and MAPK regulation. Additionally, betulinic acid enhances the effects of ionizing radiation and chemotherapeutic agents, reinforcing its potential for combination therapies aimed at overcoming resistance.

Despite its strong apoptotic activity, betulinic acid paradoxically transiently activates the EGFR/Akt-mediated survival pathway, contributing to melanoma resistance. This suggests that incorporating EGFR inhibitors into betulinic acid-based regimens could improve therapeutic outcomes. Another contradictory finding is the simultaneous increase in apoptosis and upregulation of the anti-apoptotic protein Mcl-1, likely representing a short-term cellular defense mechanism against apoptotic stimuli. Considering that Mcl-1 is regulated by both MAP-kinase and PI3-kinase/Akt pathways, targeted therapeutic interventions using anti-Mcl-1 strategies could help maximize the efficacy of betulinic acid.

The anti-metastatic properties of betulinic acid further expand its therapeutic promise. Its ability to suppress epithelial-to-mesenchymal transition suggests that it can reduce melanoma invasiveness by downregulating mesenchymal markers while reinforcing epithelial characteristics. Additionally, betulinic acid exhibits synergistic effects when combined with vincristine, leading to greater inhibition of metastatic progression. Its role in mitochondrial modulation, particularly improving respiratory function, may also contribute to its broader anti-melanoma effects and ability to counteract therapeutic resistance in advanced cases.

However, certain limitations must be considered. Variability in the cytotoxic efficacy of betulinic acid across melanoma models, inconsistencies in bioavailability, and tumor heterogeneity present challenges that require optimization of delivery systems. Additionally, melanin content appears to play a role in betulinic acid resistance, with amelanotic melanoma cells demonstrating higher sensitivity than their melanotic counterparts. This observation suggests the need for personalized treatment strategies based on melanoma pigmentation and metabolic characteristics.

Carnosic acid

In vitro

Examining the impact of carnosic acid on the metastatic properties of B16F10 cells revealed its ability to inhibit cell migration in a dose-dependent manner. It also reduced cell adhesion while downregulating the secretion of vascular cell adhesion molecule (VCAM)-1, urokinase plasminogen activator (uPA), tissue inhibitor of metalloproteinase (TIMP)-1, and matrix metalloproteinase (MMP)-9. Conversely, TIMP-2 secretion increased significantly in response to carnosic acid treatment. Additionally, carnosic acid suppressed mesenchymal markers such as N-cadherin, vimentin, Slug, and Snail while inducing the epithelial marker E-cadherin, suggesting inhibition of epithelial-mesenchymal transition. Furthermore, carnosic acid reduced the phosphorylation of Akt, Src, and focal adhesion kinase (FAK).[80]

Treating B16F10 cells with carnosic acid significantly suppressed melanoma cell growth, upregulated p21 expression, and induced G0/G1-phase cell cycle arrest. It also enhanced the cell cycle-arresting and cytotoxic effects of the chemotherapeutic agents lomustine and carmustine, further amplifying their efficacy against melanoma cells.[81]

In another investigation, malignant melanoma cell lines exhibited detectable levels of NAD(P)H quinone oxidoreductase 1 (NQO1), and β-lapachone demonstrated cytotoxic effects dependent on NQO1 activity. Inhibition or reduced expression of NQO1 diminished β-lapachone-mediated toxicity. Notably, carnosic acid induced NQO1 expression via nuclear factor erythroid 2–related factor 2 (Nrf2) stabilization, significantly enhancing the cytotoxic effects of β-lapachone across all tested melanoma cell lines.[82]

In radio-resistant B16F10 cells, carnosic acid exhibited a paradoxical radiosensitizing effect. Carnosic acid treatment reduced melanoma cell survival following radiation exposure while significantly increasing radiation-induced cell death compared to irradiated cells. Additionally, carnosic acid enhanced apoptosis and further amplified cell death in bystander melanoma cells affected by radiation-induced signaling. Moreover, carnosic acid decreased the intracellular glutathione (GSH)/glutathione disulphide (GSSG) ratio, impairing antioxidant defenses and causing melanoma cells to be more susceptible to radiation-induced damage.[83]

It has been shown that carnosic acid exerted anticancer effects in melanoma cells by downregulating β-catenin expression. It significantly inhibited proliferation in B16F10 cells, reduced viability across melanoma cell lines, and induced apoptosis and cell cycle arrest in a β-catenin-dependent manner. Using CRISPR-Cas9 knockout models, researchers demonstrated that the effects of carnosic acid were mediated through the wingless/integrated (Wnt)/β-catenin pathway. Furthermore, carnosic acid exhibited the highest binding affinity for β-catenin among its analogs, correlating with increased cytotoxicity. To improve delivery, carnosic acid was incorporated into a RADA-rich peptide-based hydrogel (Supra-gelδCA), which demonstrated injectability, controlled release, and self-healing properties.[84]

It has been demonstrated that carnosic acid effectively inhibited melanogenesis in melanoma cells while suppressing melanosome transfer to keratinocytes. Moreover, carnosic acid downregulated key genes associated with melanin production, including microphthalmia-associated transcription factor (MITF), tyrosinase enzyme (TYR), and tyrosinase-related protein-1 (TRP-1), as well as genes involved in melanosome transport, such as melanophilin (MLPH), myosin (Myova), and Rab27a.[85]

In vivo

In mice with melanoma, carnosic acid treatment effectively inhibited tumor growth while lowering serum levels of liver enzymes, ALT (alanine transaminase) and AST (aspartate aminotransferase), suggesting a potential safety profile.[81]

It has been shown that following the intravenous injection of B16-BL6 cells into C57BL/6 mice, treatment with carnosic acid resulted in a significant reduction in lung weight and tumor cell colony formation. It also downregulated α4 integrin expression in B16-BL6 cells and impaired α4 and α9 integrin-dependent adhesion [86] (Table 3).

Briefly, carnosic acid exhibits a multi-dimensional role in melanoma therapy by selectively modulating key pathways that influence tumor proliferation, migration, apoptosis, and resistance mechanisms. Its ability to impair epithelial-mesenchymal transition and suppress metastatic potential highlights its relevance as an anti-invasive agent. Additionally, carnosic acid enhances the efficacy of chemotherapeutic agents, amplifies radiosensitivity in resistant melanoma cells, and disrupts crucial signaling cascades, including the Wnt/β-catenin and Akt pathways.

One of the most striking aspects of carnosic acid is its paradoxical radiosensitizing effect. Unlike conventional agents that generally enhance radiation-induced cytotoxicity across all cells, carnosic acid appears to protect normal cells while sensitizing melanoma cells, suggesting a selective therapeutic advantage in radiotherapy. This dual role could contribute to reducing collateral damage to surrounding healthy tissue, possibly lessening side effects in cancer patients undergoing radiation treatment.

However, certain contradictions appear. While carnosic acid facilitates apoptosis and enhances cytotoxicity, its impact on melanoma cell adhesion varies depending on

Table 3. Effects of carnosic acid and carnosol on melanoma

Compound	Study design	Doses/Duration	Results	Ref.
		Carnosic acid		
		In vitro		
Carnosic acid	B16F10 cells	2.5–10 µmol/l	↑ TIMP-2 secretion ↓ Cell migration, cell adhesion, VCAM-1, uPA, TIMP-1, MMP-9, N-cadherin, vimentin, slug, snail, phosphorylation of Akt, Src, and FAK	80
Carnosic acid	B16F10 cells	0, 5, 10, 15, 20, 25, 50, and 100 µM, 24 h	↑ G0/G1 phase cell cycle arrest, p21 expression, cytotoxicity ↓ Cell growth	81
Carnosic acid	B16BL6, CRL-1585, G-361, HMV-II, GAK, HMY-1, MeWo, SK-MEL-2, SK-MEL-31, MM-AN, and SK-MEL-28 cells	0.5 or 40 µM, 24 h	↑ NQO1 expression, Nrf2 stabilization, activation of the KEAP1- Nrf2, sensitivity to β-lapachone	82
Carnosic acid	B16F10 cells	25 µM, 48 h	↑ Geno-protective effects in irradiated cells, apoptosis ↓ Cell survival, GSH/GSSG ratio	83
Carnosic acid	B16F10, A-375, and SK-MEL-2 cells	-	↑ Anticancer effects, apoptosis, binding affinity for β-catenin, cell cycle arrest, cytotoxicity ↓ β-catenin expression, viability	84
Carnosic acid	B16F10 cells	5, 10, 20, 40, and 80 µM, 42 h	↓ Melanogenesis, melanosome transfer to keratinocytes, tyrosinase activity, MITF, TYR, TRP-1, MLPH, Myova, Rab27a	85
		In vivo		
Carnosic acid	Male C57BL/6 mice	50 mg/kg, every 2 days, 14 days, i.p.	↓ Tumor growth	81
Carnosic acid	C57BL/6 mice	-	↓ Lung weight, tumor cell colony formation, α4 integrin expression in B16-BL6 cells, α4 and α9 integrin-dependent adhesion	86
		Carnosol		
		In vitro		
Carnosol	B16F10 cells	1.25, 2.5, 5, 10, 15, and 20 µM, 0-24 h	↓ Tumor cell invasion, metalloproteinase activity, MMP-9, at tyrosine phosphorylation of ERK1/2, Akt, p38, and JNK, NF-κB, AP-1	87
Carnosol	B16F10 cells	10, 20, and 40 µM, 24 and 48 h	↑ Radio-sensitizer effect, radiation-induced cell death, cytotoxicity	88
Carnosol	G361 cells	20, 50, and 100 µM, 12-72 h	↑Apoptosis, ROS, Bax expression, p53 protein levels ↓ Bcl-2 and Bcl-xL, MDM2, Src and STAT3 activation, expression of D-series cyclin and survivin	89

Akt: protein kinase B; AP-1: activator protein-1; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; Bcl-xL: B-cell lymphoma-extra large; ERK: extracellular signal-regulated kinase; FAK: focal adhesion kinase; GSH: glutathione; GSSG: glutathione disulfide; JNK: c-Jun N-terminal kinase; KEAP1: Kelch-like ECH-associated protein 1; MDM2: mouse double minute 2 homolog; MITF: microphthalmia-associated transcription factor; MLPH: melanophilin; MMP-9: matrix metallopeptidase-9; Myova: myosin; NF-κB: nuclear factor-kappa B; NQO1: NAD(P)H quinone dehydrogenase 1; Nrf2: Nuclear factor erythroid 2-related factor 2; ROS: reactive oxygen species; STAT3: signal transducer and activator of transcription 3; TIMP: tissue inhibitor of metalloproteinases; TRP-1: tyrosinase-related protein-1; TYR: tyrosinase; uPA: urokinase plasminogen activator; VCAM-1: vascular cell adhesion molecule-1.

context—some findings suggest suppression of adhesion markers, while others indicate integrin modulation without complete loss of attachment ability. This discrepancy may reflect differences in experimental conditions, cell lines, or drug concentrations, necessitating further investigation into optimal dosing and formulation approaches. Furthermore, despite strong preclinical evidence, its pharmacokinetic and biodistribution profiles *in vivo* remain limitations that require refinement, particularly for systemic therapeutic applications.

To maximize its potential, innovative drug delivery strategies such as nano-formulations or peptide-based hydrogels (like the RADA-rich Supra-gelδCA) could enhance bioavailability and improve controlled drug release. Combining carnosic acid with existing chemotherapeutics or radiation procedures in a sequential or synergistic approach may optimize melanoma treatment outcomes. Further studies integrating transcriptomic and proteomic analyses could elucidate its precise molecular interactions, strengthening its translational prospects for clinical applications.

Carnosol

In vitro

Treating B16F10 cells with carnosol inhibited tumor cell invasion in a dose-dependent manner. Also, a significant reduction in metalloproteinase activity was observed, in metalloproteinase activity, particularly MMP-9, at both protein and messenger ribonucleic acid (mRNA) levels. In addition, carnosol attenuated the tyrosine phosphorylation of key signaling molecules, including Akt, ERK1/2, JNK, and p38, alongside inhibition of transcription factors nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1).[87]

The findings of a study illustrated that carnosol exhibited a notable protective effect in normal prostate epithelial cells, reducing radiation-induced cell death. Conversely, in melanoma cells, it functioned as a radiosensitizer, increasing radiation-induced cell death and enhancing susceptibility to damage.[88]

Exploring the apoptotic and anticancer mechanisms of carnosol in G361 cells revealed that carnosol induced apoptosis in G361 cells in a time- and dose-dependent manner, accompanied by a significant increase in intracellular ROS. Carnosol reduced the levels of anti-apoptotic proteins Bcl-2 and B-cell lymphoma-extra large (Bcl-xL) while upregulating the pro-apoptotic protein Bax. As well, it elevated p53 protein levels while downregulating mouse double minute 2 homolog (MDM2). Carnosol further inhibited the activation of signal transducer and activator of transcription 3 (STAT3) and Src, leading to decreased expression of STAT3-dependent genes such as survivin and D-series cyclin. Notably, pre-treatment with N-acetyl cysteine attenuated these effects [89] (Table 3).

To sum up, carnosol exhibits a complex role in melanoma treatment, primarily through its ability to induce apoptosis, modulate signaling pathways, and influence redox homeostasis (Figure 2). A key mechanism involves the generation of ROS, which disrupts cellular balance and leads to enhanced melanoma cell death. Moreover, carnosol inhibits critical oncogenic pathways, including STAT3 and NF-κB, thereby reducing melanoma cell survival and proliferation. Suppression of metalloproteinase activity, particularly MMP-9, further contributes to its anti-invasive properties, suggesting its potential to limit metastatic progression.

Remarkably, while carnosol demonstrates radioprotective effects in healthy cells, in melanoma cells, it appears to act as a radiosensitizer, intensifying radiation-induced cytotoxicity. This dual action highlights a significant therapeutic advantage, as it may enhance the efficacy of radiation therapy while sparing normal tissues from damage. However, the absence of *in vivo* and clinical evidence limits the translation of these findings into practical applications. Further exploration is necessary to determine its systemic effects, optimal dosing, and potential interactions with existing therapeutic modalities.

Rosmarinic acid

In vitro

Rosmarinic acid exhibited a protective effect against oxidative stress in melanoma cells, mitigating the cytotoxicity induced by hydrogen peroxide (H_2O_2). Exposure to H_2O_2 led to a dose- and time-dependent decline in cell adhesion. Rosmarinic acid treatment notably improved cell adhesion compared to H_2O_2-exposed groups. Similarly, H_2O_2-induced LDH activity increased proportionally, reflecting heightened membrane damage, whereas rosmarinic acid administration significantly reduced LDH activity. Furthermore, rosmarinic acid exhibited substantial 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effects, comparable to vitamin E, reinforcing its antioxidant effect.[90]

In normal prostate epithelial cells (PNT2), rosmarinic acid improved cell survival post-X-ray exposure, reducing radiation-induced cell death. Conversely, in B16F10 melanoma cells, rosmarinic acid functioned as a radiosensitizer, increasing cellular mortality.[91]

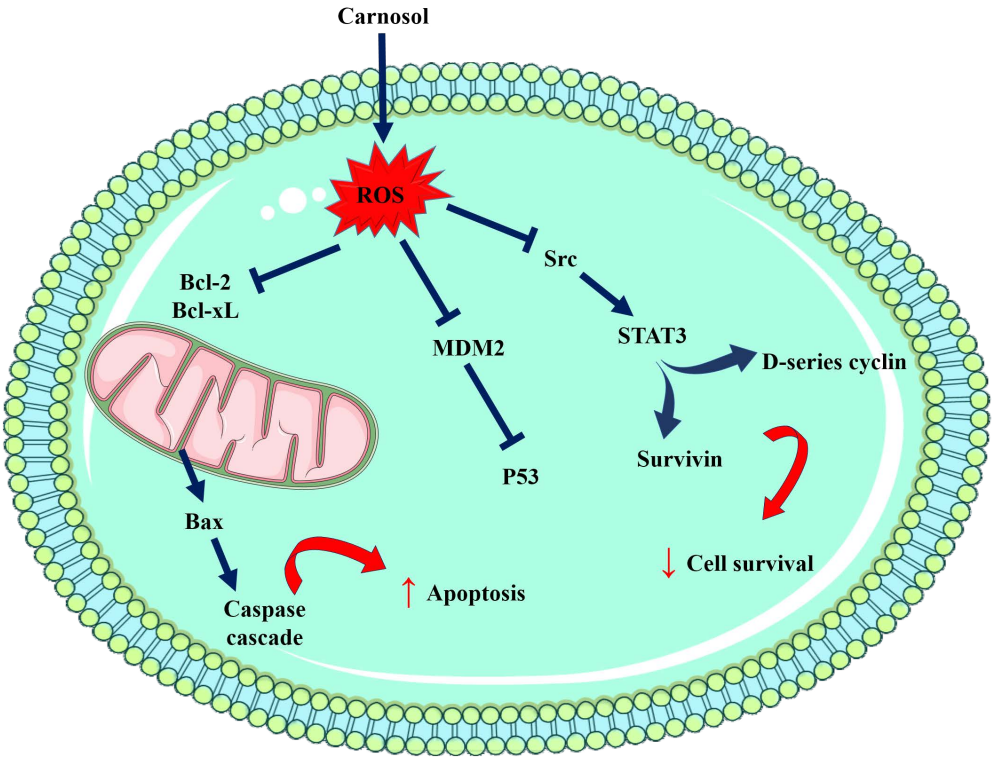


Figure 2. Mechanistic overview of anti-melanoma effects of carnosol (Images from https://smart.servier.com)

The paradoxical radiosensitizing effect of rosmarinic acid on B16F10 cells was explored by analyzing intracellular GSH production in these radio-resistant cells, compared to radiosensitive PNT2 cells. In PNT2 cells, rosmarinic acid administration significantly increased total GSH levels and elevated the GSH/GSSG ratio in irradiated cultures. On the contrary, in B16F10 cells, rosmarinic acid had no effect on total intracellular GSH levels but decreased the GSH/GSSG ratio; in irradiated melanoma cells, this reduction was more pronounced, signifying heightened radio-induced damage.[92]

A375 cells were treated with rosmarinic acid, leading to a marked reduction in cell invasion, migration, proliferation, viability, and melanin production. The treatment also suppressed the expression of MMP-2 and MMP-9 while enhancing apoptosis, as indicated by Bcl-2 downregulation and upregulation of pro-apoptotic proteins. Furthermore, rosmarinic acid augmented cytotoxic effects of cisplatin and significantly inhibited the a disintegrin and metalloprotease 17 (ADAM17)/EGFR/Akt/glycogen synthase kinase 3 Beta (GSK3β) signaling axis, with additional suppression observed upon co-treatment with the ADAM17 inhibitor (TACE prodomain (TPD)). Notably, ADAM17 overexpression contradicted the anti-tumor effects of rosmarinic acid, whereas TPD reinforced its inhibitory role.[93]

In another study, SK-MEL-28 cells were treated with rosmarinic acid, leading to a substantial reduction in cell migration and viability and. Further analysis confirmed rosmarinic acid-induced mitochondrial transmembrane potential loss and apoptotic body formation. Rosmarinic acid also decreased intracellular and extracellular ROS levels while enhancing antioxidant defenses, including non-protein thiols (NPSH) and total thiols (PSH). Furthermore, rosmarinic acid upregulated the gene expression of caspase-3 and caspase-8 while downregulating the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) inflammasome, with a notable increase in caspase-3 enzymatic activity.[94]

Similarly, SK-MEL-28 cells were exposed to varying rosmarinic acid concentrations, resulting in significant apoptosis induction. Additionally, rosmarinic acid downregulated the expression of CD73 and A2A receptors, particularly at higher treatment concentrations. Furthermore, rosmarinic acid enhanced cytokine production, increasing levels of tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-4, IL-6, and IL-10, which may contribute to its immunomodulatory effects [95] (Table 4).

A Quality by Design-guided study developed a rosmarinic acid-loaded cubosomal hydrogel for topical melanoma therapy and enhanced dermal delivery. Electron microscopy confirmed the formation of uniform spherical cubosomes with preserved lipid bilayer architecture. Incorporation of the nanoparticles into a xanthan gum-based hydrogel improved drug delivery performance, producing higher cumulative drug release at 12 h (79.2%) compared with a plain rosmarinic acid hydrogel (64.7%). In A-375 human melanoma cells, the rosmarinic acid-loaded cubosomal hydrogel exhibited significant antiproliferative activity, with an IC_{50} value of 18.14 μg/ml, while confocal microscopy confirmed efficient cellular uptake and mitochondrial localization.[96]

In brief, rosmarinic acid demonstrates favorable anti-melanoma potential by influencing multiple protective mechanisms (Figure 3), including apoptosis induction, oxidative stress modulation, immune signaling, and cell migration suppression. The paradoxical role of rosmarinic acid in oxidative stress regulation across different studies may stem from the complex interplay between its pro-oxidant and antioxidant effects, which vary based on concentration, cellular redox status, and treatment conditions. In certain studies, rosmarinic acid lessens oxidative stress by scavenging free radicals and upregulating antioxidant defenses, such as thiol compounds, while

Table 4. Effects of rosmarinic acid on melanoma

Compound	Study design	Doses/Duration	Results	Ref.
		In vitro		
Rosmarinic acid	SK-MEL-3 cells	100 to 130 μM	↑ Cell adhesion, DPPH radical scavenging capacity ↓ LDH activity, oxidative damage	90
Rosmarinic acid	B16F10 cells	10, 20, and 40 μM, 24 and 48 h	↑ Radiosensitizing, cellular mortality	91
Rosmarinic acid	B16F10 cells	30 μM, 1-3 h	↑ Radio-induced damage ↓ GSH/GSSG ratio	92
Rosmarinic acid	A375 cells	0, 50, 100, and 200 μg/ml, 24-72 h	↑ Apoptosis, pro-apoptotic proteins, cytotoxic effects of cisplatin ↓ Cell viability, invasion, migration, proliferation, melanin production, MMP-2 MMP-9, Bcl-2, ADAM17/EGFR/AKT/GSK3β signaling axis	93
Rosmarinic acid	SK-MEL-28 cells	24 h	↑ Apoptotic body formation, antioxidant defenses, expression of caspase-3 and caspase-8, caspase 3 enzymatic activity ↓ Cell migration and viability and migration, intracellular and extracellular ROS levels, NLRP3 inflammasome	94
Rosmarinic acid	SK-MEL-28 cells	-	↑ Apoptosis, TNF-α, IFN-γ, IL-4, IL-6, IL-10 ↓ CD73 and A2A receptors	95

ADAM17: a disintegrin and metalloprotease 17; Akt: protein kinase B; Bcl-2: B-cell lymphoma 2; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; EGFR: epidermal growth factor receptor; GSH: glutathione; GSK3β: glycogen synthase kinase 3 Beta; GSSG: glutathione disulphide; IFN-γ: interferon-gamma; IL: interleukin; LDH: lactate dehydrogenase; MMP: matrix metalloproteinase; NLRP3: nucleotide-binding domain, leucine-rich repeat-containing family, pyrin domain-containing 3; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-alpha.

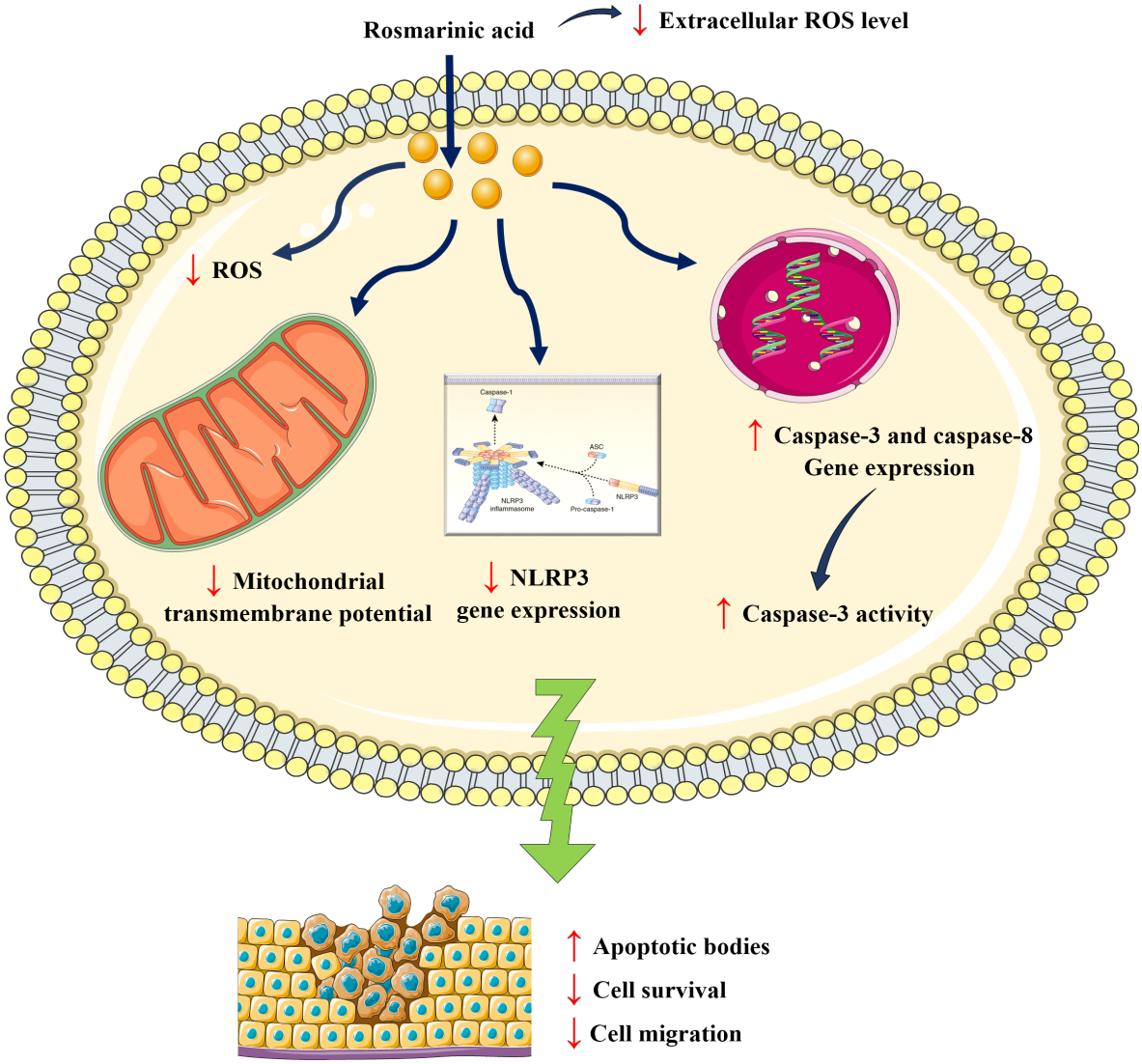


Figure 3. Schematic representation of anti-melanoma mechanisms of rosmarinic acid (Images from https://smart.servier.com and https://commons.wikimedia.org/wiki/)

in others, it promotes oxidative imbalance, possibly to induce apoptosis in melanoma cells. This dual behavior may be attributed to differential metabolic adaptations in melanoma subtypes, where redox homeostasis determines treatment sensitivity. One possible explanation is that rosmarinic acid activates the eumelanin synthesis pathway in melanoma cells, leading to intracellular GSH depletion and diminishing its protective function against oxidative stress, thereby sensitizing tumor cells to oxidative damage.

Beyond its oxidative effects, rosmarinic acid plays a crucial role in tumor suppression by targeting apoptotic pathways and immune signaling. It has been shown to promote apoptosis while modulating purinergic signaling, potentially reversing immune suppression and enhancing anti-tumor activity. Besides, one of the most interesting aspects of rosmarinic acid action is its ability to sensitize melanoma cells to conventional therapies, such as cisplatin and radiotherapy, by interfering with survival pathways like ADAM17/EGFR/Akt/GSK3β signaling and downregulating CD73/A2A receptors. These effects suggest rosmarinic acid could serve as an adjuvant to existing treatments, enhancing efficacy while potentially minimizing resistance mechanisms. However, while its immunomodulatory effects—such as cytokine upregulation—add another layer to its therapeutic potential, the absence of *in vivo* and clinical validation remains a significant limitation.

Another important contradiction within the findings relates to the influence of rosmarinic acid on apoptosis and oxidative stress. While one study reported that rosmarinic acid decreased oxidative stress while promoting apoptosis, others suggest that rosmarinic acid-induced oxidative damage contributes to cell death. These discrepancies may be explained by variations in cell lines, treatment duration, or microenvironmental conditions. Future research should aim to clarify whether the pro-apoptotic and redox-modulating effects of rosmarinic acid are context-dependent or if a general mechanism governs its function in melanoma.

The key limitation across all studies is the lack of preclinical and clinical validation, which prevents direct translational application. While rosmarinic acid exhibits

multi-targeted therapeutic benefits, factors such as bioavailability, systemic effects, and safety profiles need further investigation in animal models and human trials. If optimized, rosmarinic acid holds potential as an adjunct or standalone therapeutic for melanoma, particularly in overcoming drug resistance and modulating immune suppression. Future research should focus on refining rosmarinic acid-based delivery systems, such as nano-formulations, to enhance stability and selective tumor targeting.

Ursolic acid

In vitro

Ursolic acid exhibited potent anti-proliferative effects in B16 melanoma cells through inhibiting cell growth, altering cell cycle progression, particularly inducing an early arrest at the G1 phase.[97]

Treating M4Beu melanoma cells with ursolic acid demonstrated significant antiproliferative effects, mediated by caspase-3 activation. Ursolic acid triggered cell death through the mitochondrial intrinsic pathway, as evidenced by mitochondrial membrane potential collapse ($\Delta\Psi m$), an altered Bax/Bcl-2 balance with upregulated Bax and downregulated Bcl-2, and the release of apoptosis-inducing factor (AIF) from mitochondria.[98]

In vitro assays demonstrated that β-ursolic acid effectively inhibited serine proteases (urokinase, thrombin, and trypsin) and the cysteine protease cathepsin B. Notably, it exhibited the strongest inhibitory activity against cathepsin B and urokinase, both of which are implicated in tumor invasion and metastasis.[99]

On B16F10 cells, ursolic acid treatment at non-toxic concentrations led to the formation of apoptotic bodies and dose-dependent DNA fragmentation. Molecular analysis showed the upregulation of apoptotic genes p53 and caspase-3, alongside the downregulation of the anti-apoptotic gene bcl-2, indicating enhanced apoptotic activity. Furthermore, key transcription factors (activating transcription factor-2 (ATF-2), c-FOS, cAMP responsive element binding protein 1 (CREB-1), NF-κBp65, NF-κBp50, and NF-κBc-Rel) were significantly inhibited in ursolic acid-treated cells compared to controls. Ursolic acid also suppressed pro-inflammatory cytokine production, reducing TNF-α, IL-1β, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF) expression levels in treated melanoma cells.[100]

A research examined the interplay between melanogenesis and apoptosis resistance in melanoma, focusing on the effects of ursolic acid on B16-F0 cells. Despite apoptotic induction of ursolic acid, melanoma cells exhibited the ability to delay cell death. Remarkably, upregulation of tyrosinase-related protein (TRP)-1 and tyrosinase in apoptotic cells led to increased melanin production, which appeared to contribute to apoptosis resistance. In contrast, TRP-2—typically associated with melanoma's resistance to cell death—was downregulated.[101]

Researchers examined the sensitizing and protective effects of ursolic acid against photooxidative stress using ATCC CRL-11147 cells. Exposure to broadband UV-visible radiation led to reduced proliferative capacity, NF-κB activation, and increased protein carbonylation. Pretreatment with ursolic acid significantly decreased phosphorylated NF-κB levels, while also enhancing melanoma cell sensitivity to UV radiation.[102]

Investigating the effect of ursolic acid on apoptosis regulation in melanoma cells following ultraviolet-visible radiation (UVR) exposure disclosed that ursolic acid modulated oxidative stress responses and mitochondrial apoptosis-related genes, particularly p53 and NF-κB. Ursolic acid pretreatment enhanced UVR-induced apoptosis in melanoma cells. Moreover, ursolic acid reduced nuclear translocation of p65 and p53 before UVR exposure, though melanoma cells maintained elevated p65 and p53 levels post-treatment. Furthermore, ursolic acid and rapamycin exacerbated mitochondrial membrane potential collapse upon UVR exposure, further promoting melanoma cell death.[103]

It has been observed that ursolic acid significantly suppressed melanoma cell proliferation in a concentration-dependent manner. Apoptosis induction was evidenced by an increased Annexin-V positive population and sub-G1 peak, alongside caspase-3 activation.[104]

Another research explored the potential of chloroquine to enhance the anticancer effects of ursolic acid against metastatic melanoma (B16F10 and A375 cells). The results demonstrated a strong synergistic reduction in cell viability upon combined treatment, despite a paradoxical decrease in autophagosome levels. Western blot analysis revealed an accumulation of microtubule-associated protein 1 light chain 3 Beta (LC3II) and a significant decline in beclin-1 and p62 expression.[105]

Additionally, ursolic acid and its derivative US597 effectively suppressed B16F10 melanoma cell adhesion, invasion, and migration. US597 significantly decreased the number of invaded melanoma cells after 24 hours of treatment. Wound-healing experiments confirmed that melanoma cell motility was notably impaired, with US597-treated cells failing to efficiently close the wound area. Molecular analysis further demonstrated downregulation of focal adhesion kinase (FAK), integrin α6, integrin β1, paxillin, and Src, alongside upregulation of phosphatase and tensin homolog (PTEN).[106]

Ursolic acid exhibited dose-dependent antiproliferative and apoptotic effects in M4Beu melanoma cells. Ursolic acid-induced apoptosis was confirmed through PARP cleavage and increased DNA fragmentation. Apoptotic cells upregulated melanogenesis, likely as a resistance mechanism, evidenced by elevated intracellular and extracellular melanin levels. Ursolic acid treatment also led to cyclooxygenase-2 (COX-2) upregulation and prostaglandin E2 (PGE2) release, further contributing to apoptosis resistance. Inhibiting melanogenesis with N-phenylthiourea (PTU) or blocking COX-2/PGE2 signaling with NS-398 enhanced ursolic acid-induced apoptosis, reducing cell viability and increasing DNA fragmentation. Simultaneous inhibition of both pathways

significantly weakened apoptosis resistance. Ursolic acid also suppressed Akt and ERK-1/2 signaling, reinforcing its pro-apoptotic effects independently of melanogenesis and COX-2/PGE2 pathways.[107]

Similarly, ursolic acid exhibited a significant dose-dependent anti-proliferative effect on SK-MEL-2 melanoma cells, primarily by inducing cell cycle arrest in the S phase. Ursolic acid also reduced cell adhesion to intercellular adhesion molecule (ICAM), while showing no impact on vascular cell adhesion molecule (VCAM).[108]

Ursolic acid exhibited dose- and time-dependent cytotoxic effects on G361 melanoma cells. Lower concentrations showed minimal impact, whereas higher concentrations inhibited proliferation. Higher concentrations markedly reduced cell density across all time points, with extended exposure intensifying cytotoxicity. DNA synthesis was also notably suppressed. Apoptosis induction was confirmed through increased caspase-3 activity. Cellular death analysis revealed apoptotic morphological changes at higher concentrations.[109]

Another group of researchers evaluated the cytotoxic effects of ursolic acid- 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) conjugates on melanoma cells. Several ursolic acid-DOTA derivatives were synthesized using different spacer units and protecting groups, with conjugates 22 and 24 exhibiting the highest cytotoxicity against A375 melanoma cells. Further analysis confirmed apoptosis induction in A375 cells treated with 24 being annexin V-positive after 24 hours. Flow cytometry revealed a substantial shift to the subG1 phase. Extended treatment with conjugate 22 (48 hours) resulted in membrane rupture, necrotic/late-stage apoptosis, and a flattened DNA distribution profile.[110]

Ursolic acid and its 3-O-acetyl derivative exhibited comparable growth inhibition 50% (GI_{50}) values and effectively induced apoptosis by activating caspases-3 and caspase-7 while upregulating Bax and downregulating Bcl-2. Ursolic acid promoted an increase in the sub-G1 cell population, whereas 3-O-acetylursolic acid induced S-phase arrest and pronounced morphological changes.[111]

It has also been illustrated that ursolic acid suppressed mitochondrial respiration, inducing a metabolic switch toward glycolysis, which enhanced the cytotoxic effect of dichloroacetate, a pyruvate dehydrogenase kinase inhibitor.[112]

Another study was designed to evaluate the effect of polylactic acid-hydroxyacetic acid copolymer nanoparticles co-loaded with ursolic acid and dacarbazine on melanoma cell viability, apoptosis, and migration. Initial assessments confirmed the synergistic effects of ursolic acid and dacarbazine. B16 melanoma cells treated with ursolic acid, dacarbazine, nanoparticles, and red blood cell membrane-coated nanoparticles (RNPs) exhibited dose- and time-dependent reductions in viability, with nanoparticles and RNPs showing the most pronounced effects. Migration assays revealed that RNPs inhibited tumor cell movement more efficiently than other treatments, while colony formation analysis demonstrated comparable antiproliferative efficacy of RNPs relative to the other groups. Further, apoptosis evaluation indicated substantial cellular destruction induced by nanoparticles, RNPs, and the ursolic acid-dacarbazine combination. Moreover, ferroptosis inhibitors (Ferrostatin-1 and Deferoxamine) partially reversed the antitumor effects of ursolic acid. RNPs significantly decreased GSH levels while elevating malondialdehyde (MDA) concentrations. Ferroptosis-associated proteins displayed distinct expression patterns, with acyl-CoA synthetase long-chain family member 4 (ACSL4) upregulated and solute carrier family 7 member 11 (SLC7A11) and glutathione peroxidase 4 (GPX4) downregulated in response to treatment. Oxidative stress analysis confirmed that nanoparticle- and RNP-treated cells generated elevated ROS. JC-1 staining showed reduced mitochondrial membrane potential in the RNP-treated cells.[113]

In vivo

An *in vivo* evaluation was conducted using a B16 mouse melanoma model, where β-ursolic acid was administered to mice. The results revealed that the compound significantly reduced lung colonization of melanoma cells.[99]

In a murine lung metastasis model, US597 significantly reduced metastatic burden in B16F10 melanoma, as evidenced by fewer and smaller metastatic nodules compared to controls. Quantitative analysis showed that US597 inhibited tumor lung metastasis, with UA treatment showing weaker inhibition. Histological examinations confirmed smaller metastatic colonies, while survival analysis demonstrated that mice treated with US597 survived over 12 weeks, compared to 8 weeks for control animals. Additionally, ICAM-1 expression in lung tissues was significantly reduced in high-dose US597-treated mice.[106]

In an *in vivo* study, B16 tumor-bearing nude mice were divided into six experimental groups. The RNPs demonstrated superior tumor growth suppression compared to all other treatment conditions, as evidenced by significantly reduced tumor size and weight. Immunohistochemical staining of Ki-67 confirmed decreased tumor cell proliferation in response to drug treatment, with the most notable reduction observed in the RNPs group. Further validation using tumor mass imaging reinforced the enhanced anticancer potential of RNPs [113] (Table 5).

In general, ursolic acid exhibits diverse therapeutic potential against melanoma by targeting tumor proliferation, apoptosis, adhesion, invasion, and metabolic regulation. Its ability to induce apoptosis is largely mediated through the mitochondrial intrinsic pathway, evidenced by Bax/Bcl-2 modulation, caspase-3 activation, and mitochondrial membrane potential collapse. Ursolic acid also disrupts focal adhesion signaling, downregulating integrin α6, integrin β1, FAK, Src, and paxillin, effectively impairing melanoma cell motility and metastatic potential.

One of the key advantages of ursolic acid is its dual functionality—it selectively sensitizes melanoma cells to

Table 5. Effects of ursolic acid on melanoma

Compound	Study design	Doses/Duration	Results	Ref.
In vitro				
Ursolic acid	B16 cells	1-20 μM	↑ Early arrest at the G1 phase ↓ Cell growth, cell cycle progression, proliferation	97
Ursolic acid	M4Beu cells	0, 10, 12.5, and 15 μM, 24 and 48 h	↑ Apoptosis, antiproliferative effects, caspase-3 activation, mitochondrial membrane potential collapse, Bax, release of AIF ↓Bcl-2	98
Ursolic acid	B16F10 cells	10–100 μM, 48 h	↑ Apoptotic bodies, DNA fragmentation, p53 and caspase-3, ↓ ATF-2, Bcl-2, c-FOS, CREB-1, NF-κBp65, NF-κBp50, NF-κBc-Rel, IL-1β, IL-6, TNF-α, GM-CSF	100
Ursolic acid	B16-F0 cells	0-15 μM, 24 and 48 h	↑ Apoptotic induction	101
Ursolic acid	ATCC CRL-11147 cells	1-2 μM	↑ Sensitivity to UV ↓Phosphorylated NF-κB	102
Ursolic acid	CRL-11147 cells	1 μg/ml	↑ UVR-induced apoptosis, p65 and p53 levels post-treatment, mitochondrial membrane potential collapse, melanoma cell death ↓ Nuclear translocation of p65 and p53 before UVR exposure	103
Ursolic acid	A375, Me4405, Mel-RM, and MM200	0-80 μM, 24 and 48 h	↑ Apoptosis, sub-G1 peak, caspase-3 activation ↓ Cell proliferation	104
Ursolic acid	B16F10 and A375 cells	0-50 μmol/l	↑ LC3II, LDH ↓ Cell viability, autophagosome levels, beclin-1, p62 expression	105
Ursolic acid and its prodrug US597	B16F10 and M619 cells	0.2-80 μM, 24 h	↑ PTEN ↓ Cancer cell migration, invasion, adhesion, integrin α6, integrin β1, FAK, Src, paxillin	106
Ursolic acid	M4Beu cells	0-17.5 μM	↑ Apoptosis, DNA fragmentation, COX-2, PGE2 ↓ Cell proliferation, cell viability, Akt and ERK-1/2 signaling	107
Ursolic acid	A2058 cells	50 and 75 μM, 48 h	↑ Pro-apoptotic properties	114
PLGA nanoparticles loaded with ursolic acid	B16F10 cells	5-100 μM, 48 h	↑ Cytotoxic effects	115
Ursolic acid	SK-MEL-2 cells	25 and 100 μM, 48 h	↑Anti-proliferative effect, cell cycle arrest in the S phase ↓ Cell adhesion to ICAM	108
Ursolic acid	G361 cells	1-20 μM, 24-72 h	↑ Caspase-3 activation, cytotoxicity, apoptotic morphological changes ↓ Cell proliferation, DNA synthesis	109
Ursolic acid and its nanoemulsion	B16 cells	-	↑ Cytotoxicity, ↓ Cell viability	116
Ursolic acid	A375 and B164A5 cells	25-100 μM, 48 h	↑ Cytotoxic effect, disrupting melanoma cell, apoptosis, cell cycle arrest in the G0/G1 phase, proliferation ↓ Bcl-2 expression	117
Ursolic acid-DOTA derivatives	A375 cells	24: 4.0 μM, 24 h 22: 3.0 μM, 48 h	- Conjugates 22 and 24 exhibited the highest cytotoxicity ↑ Apoptosis induction, substantial shift to the subG1 phase, membrane rupture, necrotic/late-stage apoptosis, flattened DNA distribution profile	110
Ursolic acid and its 3-O-acetyl derivative	A375 cells	-	↑ Apoptosis, activation of caspases-3 and caspase-7, Bax, sub-G1 cell population ↓ Bcl-2	111
Ursolic acid	WM-266-4 cells	0.002-200 μM, 4, 24, 48 h	↓ Cell proliferation	118
Ursolic acid	ED-013 (FM-55-M2), ED-013-R2, ED-094 (Mel-LE), ED-117 (Mel-NT3-00), ED-196 (Mel-DO) cells	10 μM, 24 h	↑ Metabolic switch toward glycolysis ↓ Mitochondrial respiration	112
Polylactic acid-hydroxyacetic acid copolymer nanoparticles co-loaded with dacarbazine and ursolic acid	B16 cells	Ursolic acid: 10-120 μg/ml, 24 and 48 h RNPs: 20-50 μg/ml, 24 and 48 h	↑ Anticancer activity, ferroptosis in tumor cells	113
In vivo				
β-ursolic acid	Male C57BL/6N mice	50, 75, and 100 mg/kg, 16 days, i.p.	↓ Number of B16 colonies in the lungs	99
Ursolic acid and its prodrug US597	Female B16F10/C57BL/6 mice and female Sprague–Dawley rats	20, 40, and 80 mg/kg, 28 days, p.o.	↑ Survival ↓ Metastatic burden, metastatic nodules, tumor lung metastasis, metastatic colonies, ICAM-1 expression in lung tissues	106
Polylactic acid-hydroxyacetic acid copolymer nanoparticles co-loaded with dacarbazine and ursolic acid	BALB/c mice	5 mg/kg, 4, 8, and 24 h, i.v.	↓ Tumor growth, tumor size and weight, cell proliferation	113

AIF: apoptosis-inducing factor; Akt: protein kinase B; ATF-2: activating transcription factor 2; Bax: Bcl-2-Associated X Protein; Bcl-2: B-cell lymphoma 2; COX-2: cyclooxygenase-2; CREB-1: cAMP response element-binding protein-1; DNA: deoxyribonucleic acid; ERK: extracellular signal-regulated kinase; FAK: focal adhesion kinase; GM-CSF: granulocyte-macrophage colony-stimulating factor; ICAM: intercellular adhesion molecule; IL: Interleukin; LC3II: microtubule-associated protein 1 light chain 3-II; LDH: lactate dehydrogenase; NF-κB: nuclear factor-Kappa B; PGE2: prostaglandin E2; PTEN: phosphatase and tensin homolog; TNF-α: tumor necrosis factor-alpha; UV: ultraviolet; UVR: ultraviolet-visible radiation.

UV-induced apoptosis, while protecting non-malignant cells, reinforcing its therapeutic flexibility. Moreover, ursolic acid induces metabolic shifts—suppressing mitochondrial respiration while increasing glycolysis and oxidative stress, which enhance the cytotoxic effects of metabolic modulators such as dichloroacetate. Furthermore, the ability of ursolic acid to trigger ferroptosis highlights its multi-dimensional anti-melanoma properties, as evidenced by the partial reversal of its cytotoxic effects by ferroptosis inhibitors.

Recent advancements in drug delivery strategies incorporating ursolic acid have demonstrated significant potential in enhancing its therapeutic effectiveness against melanoma. Nanoparticle-based systems, including co-loaded formulations with complementary agents, have shown improved efficacy in promoting tumor cell apoptosis and inhibiting migration. In addition, innovative delivery approaches, such as membrane-coated nanoparticles, further amplify tumor suppression by modulating oxidative stress and cellular metabolism, while influencing key molecular pathways linked to ferroptosis. These developments highlight the growing potential of targeted delivery systems to optimize the anti-cancer properties of ursolic acid and improve therapeutic outcomes in melanoma treatment.

Despite its promise, challenges remain. Melanoma cells can activate resistance mechanisms, such as melanogenesis-associated pathways and COX-2/PGE2 signaling, which counteract apoptosis. This suggests that co-targeting inflammatory and metabolic pathways could amplify the therapeutic efficacy of ursolic acid. Furthermore, variations in metastatic suppression by ursolic acid, depending on cell type and experimental conditions, highlight the need for optimized formulations, particularly nanoparticle-based approaches, to enhance bioavailability. Besides, continued preclinical and clinical evaluation will be essential to refine drug delivery strategies and maximize therapeutic potential, possibly shifting melanoma treatment patterns toward integrated combination therapies.

Future perspectives

Despite the promising anti-melanoma effects of rosemary and its main components, their clinical translation remains limited, primarily due to bioavailability challenges, tumor heterogeneity, and the complexity of melanoma resistance mechanisms. To narrow the gap, several key areas warrant further investigation.

First, advanced drug delivery systems, including nanoparticle formulations, biomimetic carriers, and controlled-release hydrogels, should be optimized to enhance systemic stability and tumor-specific targeting. RNPs and co-loaded formulations have shown impressive effectiveness, but ensuring their consistency and scalability is essential for their successful clinical application.

Second, the synergistic potential of rosemary main components with conventional therapies requires systematic evaluation. Combining rosmarinic acid, ursolic acid, carnosic acid, carnosol, and betulinic acid with chemotherapy, radiotherapy, and immune checkpoint inhibitors could amplify therapeutic responses while overcoming melanoma resistance. Moreover, combining ferroptosis-targeting strategies with apoptosis and metabolic regulation could create new therapeutic opportunities for overcoming resistant melanoma subtypes.

Third, since melanoma varies widely from patient to patient, a one-size-fits-all approach is not enough. Factors like tumor pigmentation, metabolism, and survival mechanisms all shape how a tumor responds to treatment. To improve outcomes, future research should focus on personalized medicine, using comprehensive molecular profiling to uncover biomarkers that can guide individualized therapeutic strategies.

Lastly, preclinical and clinical validation remain critical next steps. While strong *in vitro* evidence supports the anti-melanoma potential of rosemary, comprehensive *in vivo* studies and phase I/II clinical trials are essential to assess pharmacokinetics, long-term safety, and therapeutic efficacy in human patients.

Conclusion

This review highlights the significant therapeutic potential of rosemary and its main components in melanoma treatment, emphasizing their multi-targeted mechanisms spanning apoptosis induction, metabolic modulation, immune signaling, and metastatic suppression. By integrating polyphenols, flavonoids, triterpenoids, and diterpenes, rosemary exhibits synergistic anti-cancer effects, reinforcing its relevance in precision oncology.

The distinctive pharmacological profiles of rosmarinic acid, ursolic acid, betulinic acid, carnosic acid, and carnosol underscore their role in enhancing oxidative stress, disrupting focal adhesion signaling, and sensitizing melanoma cells to conventional therapies. Notably, the induction of ferroptosis through lipid peroxidation pathways further expands their potential for overcoming apoptotic resistance. Incorporating these compounds into advanced drug delivery systems and synergistic treatment strategies holds great potential for developing a natural, precise, and highly effective approach to melanoma therapy.

Despite these promising findings, several challenges remain. Limited *in vivo* and clinical studies, heterogeneous responses across melanoma subtypes, and variability in bioavailability underscore the need for further investigation. Additionally, the adaptive resistance mechanisms of melanoma, such as melanogenesis-driven survival pathways and inflammatory signaling, complicate treatment efficacy. Addressing contradictions in oxidative stress regulation, apoptosis induction, and cell adhesion responses will be crucial for optimizing therapeutic strategies.

Moving forward, the main components of rosemary, particularly rosmarinic acid, ursolic acid, betulinic acid, carnosic acid, and carnosol, show great potential for a multi-targeted approach to melanoma therapy. By integrating these compounds into advanced drug delivery systems and combination therapies, researchers may

unlock a natural, precise, and highly effective approach to treating melanoma, offering new hope for patients and advancing cancer care. Future research should focus on preclinical validation, clinical translation, and precision drug formulation, ultimately bridging experimental evidence with therapeutic application to establish rosemary main components as key constituents of modern melanoma treatment.

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