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REVIEW

The Potential of Nano-Formulated Natural Drugs in Melanoma Treatment: A Review of Pharmacological Efficacy and Mechanistic Insights

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Abstract: Melanoma is a very aggressive skin cancer; its treatment bears great challenges, hence the interest in new therapeutic approaches is growing. In this review, potential nano-formulated natural drugs from plants such as Ginseng, Pistacia lentiscus, Amaranthus hypochondriacus, and Cannabis sativa in the treatment of melanoma are discussed. We discuss various characteristics of nanoformulations, including liposomes and nanoemulsions, with respect to their ability in enhancing drug delivery and bioavailability. Key mechanisms of action including reactive oxygen species modulation, apoptotic signaling induction, immune modulation through TLR4/MyD88, and inhibition of angiogenesis by VEGF pathways are discussed. Although these natural nanoformulations show promise in improving therapeutic outcomes, challenges related to their clinical application and safety persist. Further research is warranted to fully explore how this novel approach can best be utilized against melanoma.

Keywords: melanoma, cancer, nanotechnology, natural, plants, plant-derived compounds, mechanisms of action, preclinical studies

Introduction

Melanoma is a malignant neoplasm of the melanocytes, found to be one of the most aggressive cutaneous malignancies. Its frequency has been steadily increasing throughout the world.[1] Though making up only about 1% of cases of skin cancer, melanoma accounts for a substantially large portion of skin cancer-related deaths, according to the World Health Organization.[2] Over the past few decades, melanoma incidence has been rising throughout the world; it is more common in regions of high Ultraviolet (UV) exposure.[3] This not only presents a challenge to healthcare systems but also gives rise to the need for effective prevention and treatment strategies.

The pathophysiology of melanoma involves deoxyribonucleic acid (DNA) damage from UV radiation, which has been associated with mutations in multiple key genes responsible for cell growth regulation, including BRAF, Neuroblastoma rat sarcoma (NRAS), and p53.[4,5] These mutations lead to unregulated cell proliferation, avoidance of apoptosis, and finally the formation of tumors. Melanomas usually first appear as a new nevus or an abnormal or changing nevus.[6] The border is irregular, asymmetrical, and has various colors like brown, black, blue, or even white or red.[7] The earlier symptoms of melanoma include itching, tenderness, or bleeding within the lesion, though in its early stage, it may not produce any symptom.[8] It can be an aggressive progression; melanoma burrows into deeper layers of the skin, a pathway for cancerous cells to gain access to the bloodstream and lymphatic vessels to metastasize to distant organs[6] (Figure 1). If left unnoticed and untreated, melanoma can quickly metastasize and becomes far more difficult to treat, with much poorer survival rates.

The main treatment modalities of melanoma currently include surgical excision, immunotherapy, targeted therapy, and chemotherapy.[10] Surgical resection remains a cornerstone in early-stage melanoma; however, the treatment options in metastasis become more complex.[11] Checkpoint inhibitors in immunotherapy have emerged as a breakthrough in the

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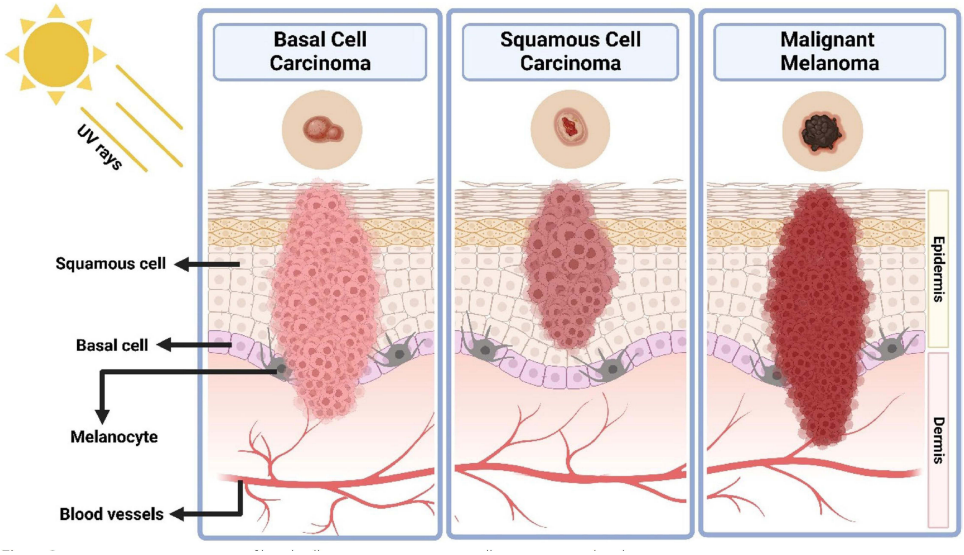

Figure 1 The figure shows the various stages melanoma progresses through: from early to advanced. Stage (**I**) During this early stage, melanoma remains confined to the epidermis, arising from melanocytes. It is usually very small and localized. Stage II: Melanoma continues to get larger and thicker at this stage, but it remains only within the epidermis. Stage III: Melanoma now breaks free and commences invasion into regional lymph nodes and soft tissues around it, as malignant cells begin their journey through the lymphatic vessels. In stage IV, melanoma finally spreads to lymph nodes, tissues, and organs far away from the primary tumor. The cancer cells could also move through the blood to distant organs like the lungs, liver, or brain. This progression emphasizes the fact that the earlier the detection, the better the prognosis of the patients. Reprinted from Zeng L, Gowda BHJ, Ahmed MG et al. Advancements in nanoparticle-based treatment approaches for skin cancer therapy. *Molecular Cancer* 2023; 22: 10. Creative Commons.[65]

treatment landscape of pembrolizumab and nivolumab and offer substantial survival benefits for patients;[12] however, these therapies are not universally effective and can be associated with severe immune-related adverse events.[13] Moreover, development of resistance toward targeted therapies, for example, BRAF and Methyl ethyl ketone (MEK) inhibitors, further complicates the therapeutic regimens and, therefore, are in dire need of new alternative therapeutic approaches able to overcome these limitations.[14]

Natural products have long played a paramount role in the discovery and development of drugs, and their importance in oncology is well appreciated.[15] Many compounds from natural sources exhibit potent anti-tumor activities; most of their mechanisms target various aspects of tumor growth and metastasis.[16] The use of natural drugs, particularly in conjunction with modern therapeutic techniques, may offer a better efficacy-toxicity ratio.[17] More recent studies have placed emphasis on the ability of phytochemicals to modulate multiple signaling pathways involved in melanoma progression, emphasizing their role as viable candidates for complementary or alternative therapies.[18,19]

The advent of nanotechnology has further accelerated interest in the potential of natural drugs. Nano-formulation of these compounds enhances bioavailability, targeted delivery, and increases the therapeutic index, thereby overcoming some of the limitations associated with conventional formulations. Encapsulation of natural products into nanocarriers is prompting investigators to come up with novel strategies for opening ways for their application in the treatment of melanoma.[20] This is a nascent area of study that not only parallels the drive toward personalized medicine but also provides new opportunities for overcoming the hurdles placed by the heterogeneous nature of melanoma.

The role of plant extracts in the development of nanoformulations is a critical aspect that significantly impacts their efficacy, stability, and safety. Plant extracts can serve diverse functions in the synthesis and stabilization of nanoparticles, acting as capping agents, emulsifiers, or reducing agents, which facilitate the formation and functionalization of nanoformulations. For instance, in the green synthesis of zinc oxide (ZnO) nanoparticles using Heliotropium indicum, plant extracts acted as capping agents, contributing to the stabilization and uniformity of the nanoparticles, as highlighted in studies on photocatalytic and photoluminescent applications.[21] Similarly, Heliotropium indicum extract was employed in the synthesis of albumin-coated ZnO nanoflowers, where it not only served as a capping agent but also enhanced biocompatibility and therapeutic efficacy against melanoma cells by inducing oxidative stress.[22] These examples

underscore the multifaceted role of plant extracts in nanoformulation development, enhancing drug delivery systems by improving nanoparticle stability, functional properties, and therapeutic outcomes.

Therefore, in light of this background, this review tries to give a detailed explanation of the pharmacological efficacy and mechanistic insights associated with nano-formulated natural drugs in the treatment of melanoma. This manuscript highlights the innovative use of nanoformulated natural drugs to overcome limitations of conventional melanoma treatments, such as poor bioavailability and systemic toxicity. By bridging traditional medicine and modern nanotechnology, it provides valuable insights and a foundation for advancing cancer therapies.

Nano-Formulation Techniques for Natural Compounds

Liposomes and Nanofibers

Liposomes and nanofibers are among the efficient delivery systems of natural compounds in melanoma by enhancing their stability, bioavailability, and targeting potential. Spherical vesicles with a structure of a lipid bilayer, liposomes are especially suited to the encapsulation of bioactive components because of their biocompatibility and capability to bear hydrophilic as well as hydrophobic agents.

Nanoemulsions and Nanoparticles

Nanoemulsions and nanoparticles are being developed as efficient carriers of natural anticancer agents, enhancing the solubility, stability, and also the targeted cellular uptake of active pharmaceutical ingredients.[23] Nanoemulsion prepared by dispersion of oils in water or water in oils at nanodimension has presented large surface area, allowing effective interaction with melanoma cells.

Self-Assembling and Bio-Mimicking Nanocarriers

Self-assembling and bio-mimicking nanocarriers therefore represent a sophisticated strategy in targeted therapy in melanoma. Self-assembling systems can self-organize as nanoparticles under physiological conditions and provide ease of encapsulation, with the ability to deliver bioactive agents in a stable manner.

Natural Medicines Used as Nanoformulations in the Treatment of Melanoma

Some of the medicinal plants appeared as promising sources for developing natural nanoformulation in the treatment of melanoma, showing their ability to enhance therapeutic efficacy with reduced side effects.[23–49] Several plant extracts, including Ginseng, Pistacia lentiscus, and Amaranthus hypochondriacus, have been incorporated to make nanoparticles that hold high anticancer activity. These nanoformulations use the bioactive compounds in the plants to inhibit melanoma cell proliferation, induce apoptosis, and modulate immune responses.[50] Better still, nanotechnology allows for the encapsulation of these natural extracts, enhancing stability, bioavailability, and their targeted transport into tumor sites (Table 1).

Ginseng

Traditional medicinal herb ginseng was already noted for its diverse therapeutic properties; thus, interest in cancer research has now focused on active compounds possessing anticancer, anti-inflammatory, and antioxidant effects.[51] Extracts from different types of ginseng plants, including Panax ginseng, Siberian ginseng, and Thai ginseng, contain bioactive molecules capable of inhibiting melanoma cell proliferation, inducing apoptosis, and modulating the immune system responses against tumors.[30,42,44,47,51,52] More recently, some studies have focused on the use of ginseng-derived nanoparticles and bioactive compounds as potential treatment options for melanoma with the aim of enhancing the immune system's antitumor response while inhibiting melanoma growth.

Jiménez Pérez et al explored the synthesis of gold and silver nanoparticles using ginseng berry extract (GBE) and evaluated their biological effects on human dermal fibroblasts and murine melanoma cells.[47] The study showed that GBE effectively reduced and capped the nanoparticles, which exhibited high antioxidant and antibacterial activity and

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Table 1 Detailed Overview of Nano-Formulation Characteristics of Natural Drugs Used in Melanoma Treatment

Reference	Author-	Nano-Formulation Type	Natural Source/ Plant Extract Used	Active Compound(s)	Size (nm)	Surface Charge (Zeta Potential)	Targeted Molecular Pathways	Mechanism(s) of Action
[27]	Alabrahim & Azzazy (2024)	Biodegradable nanofibers	Pistacia lentiscus	Essential oils, 5-Fluorouracil	290–680	Neutral	Not specified	Synergistic anticancer effect with 5-Fluorouracil; induces apoptosis
[44]	Cao et al (2019)	Ginseng-derived nanoparticles	Ginseng (Panax ginseng)	Ginsenosides	~ 344.8	Positive	Macrophage polarization	Alters macrophage polarization to inhibit tumor growth
[38]	Castañeda-Reyes et al (2021)	Liposomes	Amaranthus hypochondriacus	Squalene, soybean lunasin	115.8–163.1	Neutral	Not specified	Inhibits melanoma cell proliferation
[23]	de Menezes Furtado et al (2017)	Nanoemulsions	Tectona grandis	Not specified	18.61	Negative	Not specified	Acts as a photosensitizer in melanoma cells
[40]	Duan et al (2020)	Zinc oxide nanoparticles	Cardiospermum halicacabum	Not specified	10–20	Negative	Apoptosis pathway	Induces apoptosis in melanoma cells
[26]	Ellithy & Abdrabo (2024)	Oil-based nanoemulsions	Plant-based extract oils	Not specified	220.5 ±3.195	Not specified	Not specified	Cytotoxic effects on melanoma cells
[48]	Hu et al (2016)	Nanoparticle formulation	Bupleurum chinense, Bupleurum kaoi	Saikosaponin-d	Not specified	Not specified	Not specified	Anti-melanoma activity through saikosaponin-d
[35]	Liu et al (2021)	Nanoemulsion	Pomelo leaves	Carotenoid, chlorophyll	13.3	Neutral	Not specified	Inhibits melanoma cell viability and proliferation
[43]	Ma et al (2019)	Hydrogel scaffolds	Grape seed	Not specified	Not specified	Not specified	Ras/ERK, PI3K/ AKT	Suppresses melanoma cell growth and aids wound healing
[29]	Mohapatra et al (2023)	Ternary solid dispersion	Piper longum	Not specified	Not specified	Not specified	Apoptosis pathway	Induces apoptosis in melanoma cells
[33]	Qiu et al (2021)	Nanoemulsion	Celastrol	Celastrol	91	Negative	PD-L1, immune modulation	Boosts immune response and enhances the abscopal effect
	Sousa et al (2024)	Nanoemulsified essential oil	Melaleuca leucadendron leaves	Essential oil	137–182	Negative	Not specified	Anti-melanoma activity with photoprotective effects
[42]	Wu et al (2019)	Gold nanoparticles	Siberian ginseng	Not specified	<538	Not specified	Not specified	Inhibits melanoma cell growth
[41]	Zhou et al (2019)	Bio-mimicking nanoparticles	Tripterygium Wilfordii	Celastrol	142.7 ± 2.8	Negative	Not specified	Targeted delivery for melanoma therapy

selective cytotoxicity toward melanoma cells, suggesting their potential in the treatment of cancer and the development of cosmetic applications.

Cao et al tried to look into the immunotherapeutic potential of ginseng-derived nanoparticles (GDNPs) in repolarizing macrophages to inhibit melanoma.[44] They showed that GDNPs could induce repolarization of M2-to-M1 macrophages via Toll-like receptor 4 (TLR4) and Myeloid differentiation primary response 88 (MyD88) pathways, elevate antitumor immunity, and repress melanoma growth in vivo, therefore placing GDNPs in a position as potentially powerful agents in immunotherapy against cancer.

Wu et al synthesized gold nanoparticles from Siberian ginseng extract and then tested their effects on melanoma cells.[42] The results showed that these nanoparticles could induce apoptosis in B16 melanoma cells by increasing reactive oxygen species and changing the expression of genes involved in apoptosis, emphasizing the anticancer activity of ginseng-derived nanoparticles.

Huo et al isolated methoxyflavones from Kaempferia parviflora, commonly known as Thai ginseng, and tested these compounds for their anti-melanogenic effects on B16F10 melanoma cells. They revealed that certain methoxyflavones remarkably inhibited tyrosinase activity and melanin production, thereby singling out black ginger as a promising source of anti-melanogenic compounds for cosmetic applications.

Ma et al prepared the gene delivery vector using cationized Panax notoginseng polysaccharide for programmed Cell Death Ligand 1 (PD-L1)-targeted melanoma immunotherapy.[30] Their results demonstrated that protein-based nanoparticles (PNP) promote anti-tumor immune responses through enhancement of macrophage repolarization and dendritic cell maturation, which could significantly inhibit melanoma growth, thus showing PNP at a great potential as a safe and effective gene therapy vector.

Pistacia Lentiscus

Alabrahim and Azzazy also presented the efficiency of Pistacia lentiscus (PLEO) nanofibers in the treatment of melanoma. It was portrayed that co-loading of PLEO with 5-Fluorouracil (5FU) onto poly-ε-caprolactone nanofibers developed a biodegradable delivery system with enhanced stability and controlled release in cancer-like acidic environments.[27] The fabricated PCL-NFs showed excellent anticancer and antioxidant activities, in that PLEO and 5FU co-loaded exhibited higher potential than each compound individually against melanoma and breast cancer.

Amaranthus Hypochondriacus

Castañeda-Reyes et al prepared a liposomal formulation containing unsaponifiable matter of oil Amaranthus hypochondriacus because of its high content of squalene and loaded soybean lunasin for melanoma therapy.[38] Squalene-enriched oil of amaranth (36.64 g/100 g oil) was obtained via supercritical carbon dioxide extraction, and the authors optimized liposomes to improve the encapsulation efficiency and particle size reduction of the same. Thereafter, such lunasin-loaded liposomes exhibited significant inhibition in the viability of A-375 and B16-F10 melanoma cells when tested. Accordingly, an encapsulated form of lunasin enhanced its activity by reducing the IC_{50} value of unencapsulated lunasin by 31.81% and 41.89% in B16-F10 and A-375 cells, respectively.

Tectona Grandis

Furtado et al have explored, for the first time, the photodynamic activity of hydroalcoholic extracts from leaves of Tectona grandis, both free and as oil/water nanoemulsions, against melanoma B16 F10 cells.[23] Using a two-step formulation procedure, the authors have successfully formulated one stable nanoemulsion with about 20 nm particles, named Tectona grandis LF leaves nanoemulsion (TGE-NE), and tested this system as a drug delivery system in sensitizing B16 F10 cells to red light coming from LED sources. The free TGE and TGE-NE showed photodynamic activity, increasing their toxicity upon light exposure. Free TGE exhibited high toxicity toward melanoma cells but with significant dark toxicity toward both B16 F10 and NIH3T3 cells, whereas the TGE-NE showed reasonable photocytotoxicity with reduced toxicity towards normal cells in the dark and, therefore, could be a safer therapeutic agent.

Pomelo (Citrus Grandis)

Liu et al studied the anti-proliferative activities of nanoemulsions of chlorophyll and carotenoids of pomelo leaves (Citrus grandis) on melanoma A375 cells.[35] Studies were carried out using high-performance liquid chromatography-mass spectrometry to identify and quantify chlorophyll and carotenoids within the leaves, and the major pigment detected was chlorophyll a (2278.3 μg/g), followed by all-trans-lutein (3012.97 μg/g) among carotenoids. Both nanoemulsions showed an average particle size of around 13 nm and high encapsulation efficiencies, approximately 99%. Chlorophyll nanoemulsion has been shown to upregulate the tumor suppressor genes including p53 and p21, pro-apoptotic markers of Bcl-2-associated protein x (Bax) and cytochrome C and downregulation of anti-apoptotic proteins including Bcl-2 and cell cycle regulators of cyclin-dependent kinase 1 (CDK) 1 and 2 resulting in enhanced apoptosis via caspase activation. Carotenoid nanoemulsion also showed similar trends in modulating gene expression and apoptosis induction.[36]

Grape Seed Extract

Ma et al developed a grape seed extract-inspiring intelligent hydrogel scaffold for melanoma treatment and wound healing.[43] Grape seed extract-enriched oligomeric proanthocyanidins hydrogel acts as a natural photothermal agent that can be precisely controlled under near-infrared laser irradiation. The oligomeric proanthocyanidins (OPC) enabled hydrogel displayed tunable rheological and mechanical properties, which allowed the high-temperature activation to target the melanoma cells while supporting effective wound healing. These scaffolds, besides inhibiting melanoma growth, also favored the proliferation of fibroblasts and migration of endothelial cells. They induced angiogenesis and skin regeneration. Thus, these scaffolds were a biocompatible and multi-functional approach against melanoma and in wound therapy.

Piper Longum

Two articles currently provide the prospects of Piper longum extracts for melanoma management as standardized extracts. For the first time, Mohapatra et al prepared a novel ultradeformable nanovesicular transgelosome loaded with Piper longum extract, which showed enhanced skin penetration and reduction of tumors in melanoma-bearing mice, an effective alternative topical therapy.[28] While complementary studies were conducted by using the same research group, one of the optimizations consisted of a fourth-generation ternary solid dispersion of Piper longum extract that showed superior anticancer efficacy in melanoma models, both as a single agent and an adjuvant to dacarbazine, improved solubility and stability, and enhanced oral bioavailability.[29] Both formulations have tremendous potential for therapeutic benefits in Piper longum extract for the management of melanoma.

Melaleuca Leucadendron

In the research conducted by de Sousa et al, the photoprotective, antioxidant, and anti-melanoma activities of nanoemulsified essential oil (EO) extracted from Melaleuca leucadendron leaves were investigated. EO was obtained by hydrodistillation with a yield of 0.59% and was characterized by gas chromatography-mass spectrometry; the chemical composition was predominantly monoterpenes and sesquiterpenes. Nanoemulsions with (NE-EO) and without EO (NE-B) were prepared and showed stability with good morphology.[25] In fact, the photoprotective analysis demonstrated that the NE-EO presented higher activity than pure NE-B and free EO. Moreover, the nanoemulsified EO showed an IC (50) for the ABTS radical of 5.30 μg/mL, significantly lower than that for pure EO (40.72 μg/mL). Cytotoxicity results indicated that both EO and NE-EO were more toxic to melanoma cells B16-F10 and MeWo, than to non-tumor L-929 and NGM cells. These results indicate that NE-EO is a potential phyto-entity that might find a place in the prevention and treatment of melanoma and thus requires further investigation of its bioactive principles.

Taraxacum officinale (Dandelion)

Tettey et al worked on the preparation of gold nanoparticles (AuNPs) by aqueous leaf extract of Taraxacum officinale and indicated that the biosynthesized AuNPs could be used for anti-melanoma, tyrosinase inhibiting, and antimicrobial activities. With the growing concern about the side effects of synthetic drugs, along with the development of their

resistance, this study attempted to present a cost-effective and safer alternative through naturally derived pharmaceuticals.[49] The prepared AuNPs showed remarkable efficiency against melanoma cells, while effective tyrosinase inhibition is an essential factor in hyperpigmentation management. Also, the resulting nanoparticles possessed antimicrobial activity, which is supposed to widen their application fields in cosmetic, medical, and food industries. The present study underlined that plant origin AuNPs are promising drugs, while their clinical use is to be further studied.

Angelica Tenuissima

Park et al assessed the anti-melanogenic actions of extracts from the root of Angelica tenuissima L. and its fermentation product by Aspergillus oryzae, with respect to melanin production in B16F10 melanocytes and tyrosinase activity.[53] The current study observed that while melanin production was inhibited above the concentration of 250 μg/mL by the crude extract, A. tenuissima (AT), this was considerably enhanced through fermentation into the fermented extract, FAT. The High Performance Liquid Chromatography (HPLC) analysis showed that the active compounds of both extracts were decursin and Z-ligustilide, whose concentration increased upon fermentation. Thus, the FAT extract containing higher amounts of decursin and Z-ligustilide provided much better inhibitory effects against melanin production and tyrosinase activity. These results suggest a possible use of fermented Angelica tenuissima extracts as natural agents in the treatment of pigmentation disorders.

Cannabis Sativa

Poommarapan et al investigated the apoptosis-inducing activities of Cannabis sativa extracts on human melanoma A375 cells, focusing on bioactive phytochemicals delta-9-tetrahydrocannabinol (THC) and nonpsychoactive cannabidiol (CBD).[54] The experimental methodology included RNA sequencing and quantitative real-time PCR analyses after treatment. Apoptosis induction in A375 cells treated with THC+CBD was confirmed by the flow cytometry analysis. Upregulation of pro-apoptotic genes was determined, like DNA damage inducible transcript 3 (DDIT), nerve growth factor receptor (NGFR); genes involved in cell proliferation were downregulated, such as Cyclin E2 (CCNE2) and Proliferating cell nuclear antigen (PCNA). Besides that, the treatment hampered the phosphorylation of the ERK1/2 signaling pathway and further disturbed melanoma cell migration. These findings suggest that extracts obtained from Cannabis sativa, containing equilibrated ratios of THC/CBD, might become a promising therapeutic approach in melanoma treatment.

Orthosiphon Stamineus

Nazari et al studied the anticancer and antiangiogenic activities of the extract of Orthosiphon stamineus Benth formulated in ethanolic phospholipid vesicles (spherosomes) on melanoma growth in a murine tumor model.[54] The purpose was to formulate and characterize the nano-ethanolic spherosome system for tumor therapeutics in an enhanced manner. The formulation of the spherosome was performed by the thin-film rehydration method followed by its conversion to gel using Acrypol 1%. Characterization studies, including particle size, surface charge, and transmission electron microscopy (TEM), were performed, proving that the spherosomes had been formulated successfully. Toxicity studies of extract and its spherosome formulation were done on an endothelial cell line, EA.hy926, which indicated that these two did not produce toxicity. Preclinical in vivo studies demonstrated a profound tumoricidal effect by inhibiting the growth of tumors by 63.98% in O.S extract alone and by 87.76% in ESP-6 formulation at a dose of 3000 mg/kg. Results indicate that Orthosiphon stamineus loaded in spherosomes has considerable promise for reducing melanoma growth without inducing tumorigenesis or antiangiogenesis.

Vassobia Breviflora

Viana et al described the phytochemical and biological properties of an aqueous extract of Vassobia breviflora, a plant from the family Solanaceae, in relation to B16-F10 melanoma cells. The authors investigated the participation of the purinergic pathway.[55] Electrospray Ionization Time-of-Flight (ESI-TOF) Mass proved the existence of several bioactive compounds, such as N-methyl-(2S,4R)-trans-4-hydroxy-L-proline and calystegine B. The extract exhibited cytotoxic activity against melanoma cells in the concentration range 0.1–10 mg/mL, while DNA damage was induced only at its

highest concentration of 10 mg/mL. Different antioxidant activities were performed by DPPH and ABTS assays, determination of ROS production, and production of NO. Besides, the extract modified the ectoenzymes responsible for purinergic signaling-ectonucleoside triphosphate diphosphohydrolase and ectoadenosine deaminase-and changed the nucleosides and nucleotides levels. Molecular docking revealed that the N-methyl-(2S,4R)-trans-4-hydroxy-L-proline compound had a high affinity of binding to purinergic receptors P2X(7) and P2Y(1). The fact that these findings provide promising growth inhibitory effects in melanoma cells makes Vassobia breviflora a valuable candidate considered in cancer therapy.

Therapeutic Mechanisms and Molecular Targets

Reactive Oxygen Species Pathway

The ROS pathway is one major molecular target in melanoma therapy with nano-formulated natural compounds. Several nanoformulations, such as zinc oxide nanoparticles synthesized from Cardiospermum halicacabum, effectively enhanced intracellular ROS levels in melanoma cells, inducing oxidative stress and the induction of apoptosis.[40] High levels of ROS disrupt cellular components and activate pro-apoptotic factors and caspase proteins responsible for programmed cell death. Thus, this ROS-mediated pathway of apoptosis is a potential target, as melanoma cells have a high propensity to oxidative damage on account of rapid cell proliferation and metabolic activity (Table 1).

Apoptotic Signaling Pathways

Another potential mechanism of action involves the targeting of apoptotic signaling pathways. Carotenoid nanoemulsions, for instance, prepared from leaves of pomelo trigger the intrinsic pathways of apoptosis via upregulation of pro-apoptotic proteins such as Bax and cytochrome C, while downregulating the anti-apoptotic proteins such as Bcl-2.[35] This change in protein expression results in the activation of a caspase cascade, especially caspases-8, −9, and −3, through which an apoptotic effect is exerted in melanoma cells. This direct engagement of apoptosis-regulating molecules is important in overcoming the resistance to cell death of melanoma cells (Figure 2).

Immune Modulation Through TLR4 and MyD88

Nano-level formulations also target immune pathways to enhance anti-tumor responses. Ginseng-derived nanoparticles interact with the immune system by initiating the TLR4/MyD88 signaling pathway, which reprograms tumor-associated macrophages (TAMs) from a tumor-promoting M2 phenotype into a tumoricidal M1 phenotype.[44] Such a phenotypic shift promotes the activation of the immune system and increases ROS production, promoting melanoma cell apoptosis. These nanoformulations target the pathways of immune modulation and therefore provide two modes of action: directly attacking the tumor cells and enhancing the body's immune response against the tumor.

Angiogenesis Inhibition via VEGF Pathways

Angiogenesis inhibition is an absolute need for the restriction of melanoma growth and metastasis, and such a pathway has been targeted by many nano-formulated natural compounds. Nanoemulsions loaded with carvacrol and rosemary essential oils have been documented to downregulate the expression of vascular endothelial growth factor (VEGF), a major pro-angiogenic factor implicated in tumor blood vessel formation.[26] Thus, by downregulating VEGF and other markers promoting angiogenesis, these nano-formulations inhibit neovascularization of the tumor-thus, starving the cancer cells and reducing their proliferation and potential to metastasize.

Inhibition of Proliferative Signaling Pathways (Ras/ERK and PI3K/AKT)

These include the Ras/ERK and PI3K/AKT pathways, which also represent critical survival and proliferation pathways in melanoma cells targeted by nano-formulated therapies.[24] For example, ginger extract-loaded nanofibers inhibit these pathways, suppress melanoma cell growth, migration, and invasiveness.[24] The inhibiting of the Ras/ERK and PI3K/AKT pathways halts the signaling processes that melanoma cells depend on for rapid division and survival; this approach is a powerful strategy in controlling melanoma progression (Figure 3).

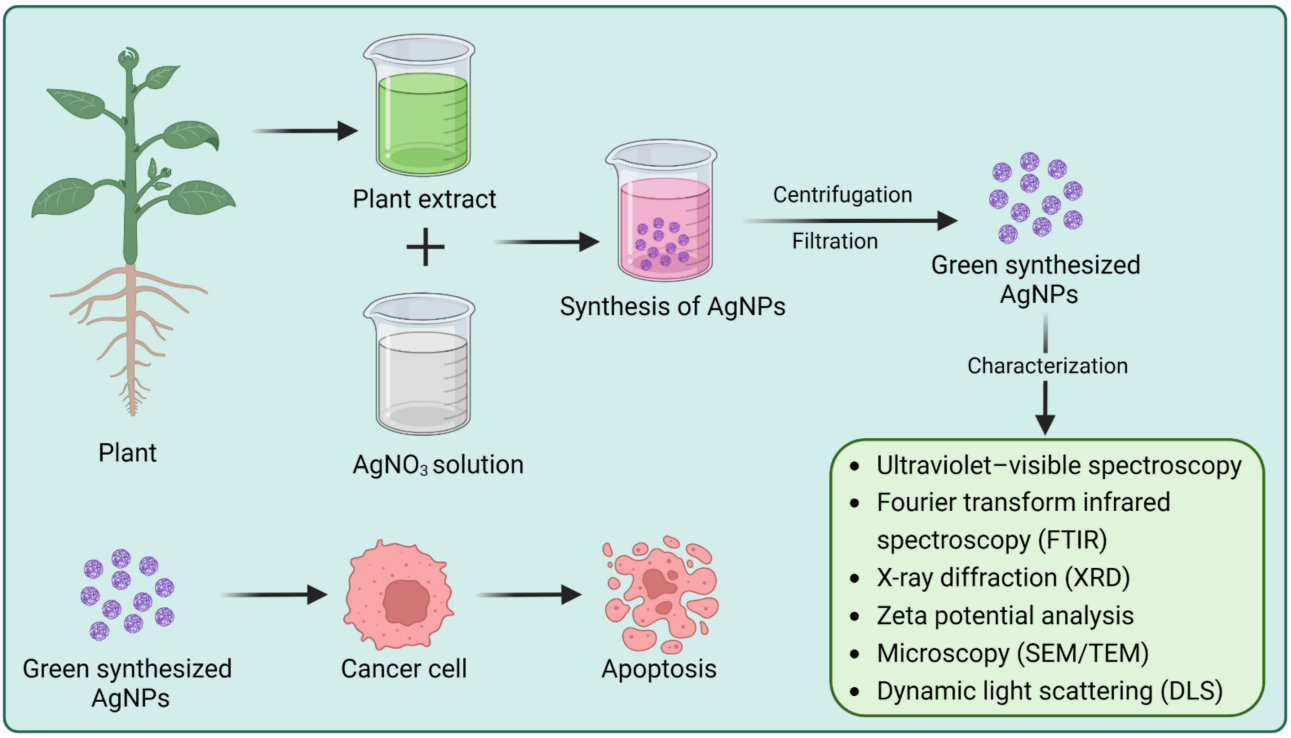

Figure 2 The green synthesis of plant-based nanoparticles against cancer: Plant extract was mixed with a metal precursor solution, centrifuged, and filtered to obtain nanoparticles. These were characterized by various techniques such as UV-visible spectroscopy, FTIR, XRD, zeta potential analysis, SEM/TEM microscopy, and DLS. Most importantly, these green-synthesized nanoparticles showed induction of apoptosis in cancer cells, which justifies their use for treating melanoma. Reprinted from Wani AK, Akhtar N, Mir TuG, et al Targeting apoptotic pathway of cancer cells with phytochemicals and plant-based nanomaterials. Biomolecules 2023; 13: 194. Creative Commons.[66]

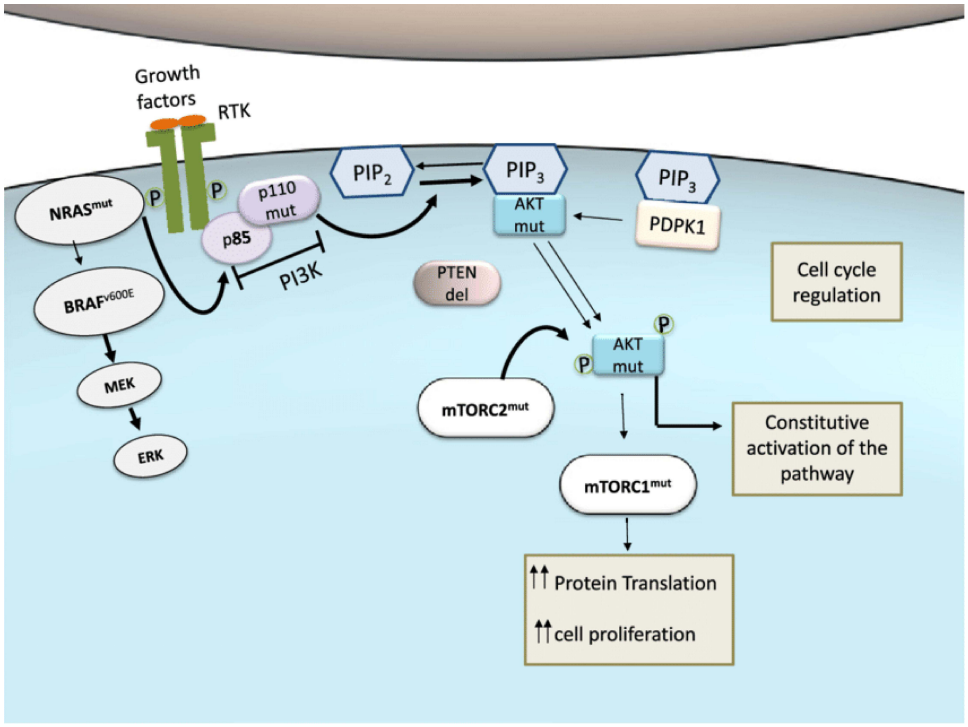

Figure 3 Activity of the PI3K-Akt pathway in melanoma. The common mutations of NRAS and BRAF oncogenes in the melanoma development promote the activity of downstream effectors along the MEK/ERK pathway. Growth factors through RTKs activate the PI3K pathway, and loss of PTEN (PTEN del) together with activating mutations of p110, AKT, and mTORC1/2 result in constitutive activation of the PI3K pathway, thus providing increased signaling through to the cell cycle regulators, protein translation, and proliferation of cells, contributing to melanoma development. Reprinted from Dantonio PM, Klein MO, Freire MRV, et al Exploring major signaling cascades in melanomagenesis: a rationale route for targetted skin cancer therapy. Bioscience reports 2018; 38: BSR20180511. Creative Commons.[67]

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Discussion

This nanoformulation significantly enhances the efficacy of natural compounds in melanoma treatment, improving their bioavailability, stability, and targeting efficiency while reducing toxic side effects. Such formulation would improve delivery of the active agent directly at the tumor site and reduce any side effects on surrounding healthy tissues. The nano-formulation offers controlled and sustained release, which increases the efficiency of treatments over some time. For example, nanoemulsions and liposomes improve the delivery of natural compounds, enhancing their poor solubility and stability; accordingly, their action against melanoma cells is considerably empowered. Their disadvantages are impairment in large-scale production, cytotoxicity at high doses, and further studies are required for long-term safety.[58] These, along with regulatory and manufacturing challenges, make translation into the clinic problematic.

Nanoformulation technology addresses key limitations of conventional drugs, such as poor solubility, rapid degradation, and systemic toxicity.[59] By encapsulating therapeutic agents in nanoscale carriers like liposomes, nanoemulsions, and polymeric nanoparticles, it enhances drug stability, bioavailability, and targeted delivery. Controlled release mechanisms minimize off-target effects and ensure sustained therapeutic action, improving treatment outcomes.[59] Additionally, nanoformulations enable drugs to bypass biological barriers, such as the tumor microenvironment, for more effective melanoma therapy.

Despite their advantages, nanoformulations face challenges that must be addressed for broader clinical adoption. One significant issue is the high production costs and difficulty in scaling up manufacturing processes.[60] Green synthesis methods using plant extracts and simpler, more cost-effective fabrication techniques can help overcome these barriers. Another challenge is the instability of many nanoformulations during storage, which can lead to aggregation or degradation.[61] Improved carrier materials, optimized formulations, and advanced storage techniques like lyophilization can enhance stability.

Immunogenicity and unexpected toxicity remain additional concerns, as some nanocarriers may trigger immune responses.[62] These risks can be mitigated through rigorous preclinical testing and modifications to nanoparticle surfaces, such as PEGylation. Regulatory hurdles also pose a barrier, with inconsistent guidelines complicating the approval process for clinical use.[63] Strengthened collaboration between researchers, industry stakeholders, and regulatory bodies is essential to streamline standards and facilitate clinical translation. Addressing these challenges will enable nanoformulation technology to achieve its full potential in transforming melanoma treatment.

The development and application of nanoformulations in melanoma treatment require navigating a complex regulatory landscape to ensure safety, efficacy, and ethical compliance. Regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established guidelines for the clinical translation of nanotechnology-based therapeutics.[64] These guidelines emphasize rigorous characterization of nanoparticles, including their size, shape, surface properties, and stability, as well as comprehensive preclinical and clinical safety evaluations.[65] Furthermore, the dual role of plant extracts in both therapeutic and nanotechnological applications adds an additional layer of complexity, as these natural compounds may require classification under multiple regulatory frameworks, such as pharmaceuticals, biologics, or dietary supplements. Harmonizing these regulations across regions remains a challenge, but initiatives such as the Nanotechnology Characterization Laboratory (NCL) and international collaboration efforts aim to streamline the approval process.[66] Addressing these regulatory hurdles is crucial for ensuring that nanoformulations not only reach clinical practice but also meet high safety and quality standards for patients with melanoma.

Advances in nanotechnology are allowing new nano-formulated therapies for melanoma treatment. Future research may be focused on developing multi-functional nano-carriers, combining imaging and therapeutic functions, which could allow for online monitoring of the therapeutic effect.[67] Biomimetic and active targeting systems, such as nanoparticles coated with cell membranes, constitute promising future strategies for the improvement of specificity and a reduction of off-target effects. Other areas of innovation include responsive nanoformulations that undergo changes in the conditions of the tumor microenvironment, such as pH or levels of enzymes, releasing their payload to optimize drug delivery to melanoma cells. Further exploration into immunomodulatory nanoformulations may also lead to new avenues for immune-based melanoma therapies.

Conclusion

The application of natural-based compounds, coupled with nanotechnological tools, offers a promising approach for advancing melanoma management. By utilizing nano-formulated natural compounds, these therapies can overcome the limitations of conventional treatments, including low bioavailability, high toxicity, and poor targeting. The integration of nanocarriers enables enhanced targeting, sustained release, and immune modulation, all of which are crucial for effectively addressing the complexities of melanoma. While challenges such as large-scale production and clinical validation persist, ongoing advancements in both natural compounds and nanotechnologies hold the potential to revolutionize melanoma treatment, providing safer, more effective, and highly targeted therapeutic options.

Disclosure

The authors report no conflicts of interest in this work.

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