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BEE VENOM IN CANCER: CELLULAR TARGETS, PRECLINICAL EVIDENCE, AND TRANSLATIONAL BARRIERS

VENINUL DE ALBINE ÎN CANCER: ȚINTE CELULARE, DOVEZI PRECLINICE ȘI BARIERE ÎN APLICARE CLINICĂ

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ABSTRACT

Objectives. Bee venom and its major peptide melittin can disrupt cell membranes and alter several cancer-related pathways, but systemic toxicity and the absence of clinical efficacy data limit translation. This review evaluated the cellular targets, preclinical evidence, delivery strategies, safety concerns, and requirements for clinical development.

Methods. A structured narrative review searched Google Scholar, PubMed Central, relevant publisher platforms and Moldovan national repositories for English-language publications available through July 6, 2026. Priority was given to mechanistic studies, normal-cell comparators, animal tumor models, targeted-delivery investigations, and clinical evidence.

Results. In preclinical models, melittin modifies tumor-cell membranes and growth-factor receptor organization; activates mitochondrial and death-receptor-mediated apoptosis; and affects phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K)/AKT/mTOR, mitogen-activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor kappa B (NF-κB), autophagy, mitophagy, endoplasmic reticulum stress, and ferroptosis-related processes. Studies also report reduced cell-cycle progression, migration, invasion, and angiogenesis, together with improved responses to selected anticancer agents. Nanocarriers reduce direct erythrocyte exposure and improve tumor delivery in animal models, but clinical antitumor benefit has not been established. No approved melittin-based anticancer therapy was identified.

Conclusions. Bee venom and melittin represent sources of bioactive compounds for anticancer drug development. However, their therapeutic potential remains preclinical, and current evidence does not support their use as alternatives to established cancer treatments. Further progress requires standardized preparations, pathway-specific validation, comparative safety studies, pharmacokinetic assessment, reproducible manufacturing, and controlled clinical trials.

Keywords: bee venom, melittin, cancer, apoptosis, autophagy, nanomedicine

REZUMAT

Obiective. Veninul de albine și peptida sa majoră, melitina, pot distruge membranele celulare și altera mai multe căi de semnalizare asociate cancerului, dar toxicitatea sistemică și absența datelor privind eficacitatea clinică limitează aplicarea clinică. Această revizuire a evaluat țintele celulare, dovezile preclinice, strategiile de administrare, problemele de siguranță și cerințele pentru dezvoltarea clinică.

Metode. O revizuire narativă structurată a inclus căutări în Google Scholar, PubMed Central, pe platformele relevante ale editorilor și în depozitele naționale din Republica Moldova pentru publicații în limba engleză disponibile până la 6 iulie 2026. S-a acordat prioritate studiilor mecaniciste, comparatoarelor pe celule normale, modelelor tumorale pe animale, investigațiilor privind eliberarea țintită și dovezilor clinice.

Rezultate. În modelele preclinice, melitina modifică membranele celulelor tumorale și organizarea receptorilor factorilor de creștere; activează apoptoza mediată de mitocondrii și de receptorii morții; și influențează procesele legate de

fosfoinozid-3-kinaza/protein-kinaza B/ținta mecanică a rapamicinei (PI3K/AKT/mTOR), proteina kinază activată de mitogeni (MAPK), kinaza Janus/traductorul de semnal și activatorul transcripției (JAK/STAT), factorul nuclear kappa B (NF- κ B), autofagie, mitofagie, stresul reticulului endoplasmatic și ferroptoză. Studiile raportează, de asemenea, reducerea progresiei ciclului celular, a migrației, invaziei și angiogenezei, împreună cu răspunsuri îmbunătățite la anumiți agenți anticancerigeni. Nanotransportorii reduc expunerea directă a eritrocitelor și îmbunătățesc livrarea la nivel tumoral în modelele animale, dar beneficiul clinic antitumoral nu a fost stabilit. Nu a fost identificată nicio terapie anticancer aprobată pe bază de melitină.

Concluzii. Veninul de albine și melitina reprezintă surse de compuși bioactivi pentru dezvoltarea de medicamente anticancer. Cu toate acestea, potențialul lor terapeutic rămâne la stadiu preclinic, iar dovezile actuale nu susțin utilizarea lor ca alternative la tratamentele oncologice consacrate. Progresele ulterioare necesită preparate standardizate, validarea specifică a căilor de semnalizare, studii comparative de siguranță, evaluare farmacocinetică, producție reproductibilă și studii clinice controlate.

Cuvinte-cheie: venin de albine, melitină, cancer, apoptoză, autofagie, nanomedicină

Introduction

Animal venoms are complex mixtures of peptides, proteins, enzymes, and low-molecular-weight compounds with diverse biological activities. Honeybee venom, produced by *Apis mellifera*, contains compounds that interact with cell membranes, ion channels, enzymes, and signaling proteins and has therefore been studied as a source of anticancer lead compounds. Its principal constituents include melittin, phospholipase A2 (PLA2), apamin, mast-cell-degranulating peptide, adolapin, hyaluronidase, histamine, and catecholamines [1-5].

Melittin is the predominant peptide component of honeybee venom and a major contributor to its acute membrane-disruptive activity. Its amphipathic, cationic structure supports binding to anionic lipid surfaces, alpha-helical insertion into phospholipid bilayers, pore formation, and membrane thinning. PLA2 hydrolyzes membrane phospholipids and can act cooperatively with melittin because peptide-induced lipid reorganization increases access to the second (sn-2) ester bond. These membrane-active effects contribute to tumor-cell killing but also cause hemolysis, pain, and injury to normal cells. The allergenic potential of whole venom is additionally associated with proteins and enzymes, particularly PLA2 and hyaluronidase [2-5].

In vitro and animal studies have reported anticancer effects in breast, lung, pancreatic, liver, colorectal, gastric, ovarian, melanoma, and hematologic models. Reported effects include growth-factor receptor inhibition, mitochondrial and death-receptor-mediated apoptosis, oxidative and endoplasmic reticulum stress, ferroptosis-related lipid damage, changes in autophagy or mitophagy, cell-cycle arrest, and reduced migration, invasion, or angiogenesis [3, 6-13]. Most evidence, however, derives from two-dimensional cell cultures or small animal studies, and clinical antitumor efficacy has not been established.

The principal translational challenge is to separate antitumor activity from systemic toxicity and to develop a reproducible, tumor-directed formulation. Some causal claims are based only on static protein measurements without functional testing, and findings obtained with whole venom are sometimes extrapolated to purified melittin. These practices limit comparison between studies and may overstate

readiness for clinical development [2, 3, 14, 15].

Whole venom, purified melittin, recombinant analogues, and nanoformulated peptides are pharmacologically distinct interventions and should be evaluated separately. Reduced protein kinase B (AKT) or mechanistic target of rapamycin (mTOR) phosphorylation after treatment does not by itself demonstrate direct kinase inhibition, because membrane permeabilization, calcium overload, and adenosine triphosphate (ATP) depletion can suppress several signaling pathways. Interpretation should therefore separate early membrane effects from downstream responses, regulated cell-death pathways from nonspecific lysis, and biomarker changes from demonstrated pathway dependence. Evidence is stronger when supported by selective inhibitors, genetic silencing, direct binding assays, normal-cell comparators, and validation in orthotopic or immunocompetent models rather than by single-time-point viability assays alone [3, 14-19].

The aim of this narrative review was to summarize evidence on the biochemical and cellular effects of bee venom and melittin in cancer, compare consistent and conflicting results, assess targeted-delivery strategies, and define the main requirements for clinical development [2, 3, 14].

Material and methods

A structured narrative-review design was used. Google Scholar, PubMed Central, and relevant publisher platforms were searched for English-language publications available through July 6, 2026. National sources were additionally searched through the Institutional Repository in Medical Sciences of Nicolae Testemițanu State University of Medicine and Pharmacy, the National Bibliometric Tool of the Republic of Moldova, and the publication platforms of Moldovan scientific institutions. Search combinations included the terms bee venom, melittin, phospholipase A2, cancer, tumor, apoptosis, autophagy, mitophagy, ferroptosis, endoplasmic reticulum stress, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), phosphoinositide 3-kinase (PI3K), AKT, mTOR, nuclear factor kappa B (NF- κ B), JAK, STAT, angiogenesis, metastasis, nanoparticle, liposome, clinical trial, and safety.

Priority was given to original studies that examined cellular pathways, *in vitro* studies using normal-cell comparators, *in*

vivo tumor models, combination studies, targeted-delivery investigations, systematic or comprehensive reviews, and assessments of clinical evidence. Publications were excluded from causal interpretation when they were unrelated to cancer, lacked sufficient methodological information, or had been retracted. Publications older than 10 years were generally excluded unless they provided foundational evidence on venom biochemistry, cell signaling or drug delivery that had not been replaced by more recent studies.

Evidence was grouped by venom component, membrane interaction, regulated cell death, cancer-related signaling, autophagy and organelle stress, tumor progression, combination therapy, drug delivery, and clinical translation. Because the review was narrative rather than systematic, no pooled effect estimate or formal risk-of-bias score was calculated. Greater weight was assigned to studies using functional validation, pharmacological rescue or genetic manipulation, normal-cell comparators, *in vivo* models, and consistent results across experiments.

No quantitative meta-analysis was performed because the studies differed substantially in tumor models, venom preparations, concentrations, exposure times, outcome measures, and combination regimens. Findings were compared for consistency, conflicting results, and strength of validation. Claims based only on metabolic viability assays, isolated increases in microtubule-associated protein 1 light chain 3 (LC3)-II or Beclin-1, or phosphorylation measurements after marked cytolysis were interpreted cautiously. The final synthesis emphasizes findings reproduced across models and the main barriers to clinical use rather than the number of positive studies.

Results

Biochemical composition and membrane-level activity

Bee venom is a variable multicomponent preparation. Its composition changes with bee subspecies, age, season, geographic origin, collection method, processing, and storage. Differences in melittin and PLA2 content can alter cytotoxicity, allergenicity, and the balance between direct membrane damage and downstream cellular responses. Studies that do not chemically characterize the venom therefore cannot be compared reliably on a mass-per-volume basis [1-5].

The interaction between melittin and PLA2 is especially relevant. Melittin disturbs lipid packing and exposes phospholipid substrates, whereas PLA2 hydrolyzes the sn-2 (second) ester bond and generates lysophospholipids and free fatty acids that further destabilize the bilayer. This cooperation can increase membrane damage and cytotoxicity compared with isolated melittin in some experimental systems, but it also increases hemolytic, inflammatory, and allergenic risk. Minor peptides may alter ion conductance or mast-cell activation, and hyaluronidase facilitates local dispersion. Comparisons between preparations therefore require reporting of bee species, collection and storage conditions, melittin content,

PLA2 activity, and endotoxin status [1-5, 15].

Melittin contains 26 amino acids arranged into a hydrophobic N-terminal region and a positively charged C-terminal region. Some tumor cells show greater externalization of phosphatidylserine, altered sialylation, increased membrane fluidity, or abnormal cholesterol organization, which can favor binding of cationic peptides. These features vary among tumors and may also occur in activated or injured normal cells; relative tumor selectivity is therefore context-dependent [1-5, 14-16, 20].

After membrane binding, melittin accumulates at the lipid-water interface and disturbs lipid packing. Depending on peptide concentration, membrane composition, and asymmetry between the two leaflets, this process may generate transient lipid-lined pores or broader bilayer destabilization rather than stable protein channels. Rapid ion flux, potassium loss, calcium entry, osmotic imbalance, and membrane depolarization can produce lytic cell death at high local concentrations. At lower concentrations, cells may remain sufficiently intact to initiate regulated stress responses. Signaling changes measured after extensive membrane damage may therefore result from ATP depletion rather than direct engagement of a specific intracellular target [2, 3, 20].

Relative tumor selectivity is based on membrane composition rather than on a tumor-specific receptor. Malignant cells may externalize phosphatidylserine, display negatively charged glycoproteins, and alter cholesterol, sphingolipid, or membrane-potential profiles, which can increase peptide binding. These properties vary among tumor clones and during treatment, while erythrocytes remain highly susceptible. Safety estimates also depend on the normal comparator used. Evaluation should therefore include cancer-cell viability, erythrocyte hemolysis, endothelial injury, plasma-protein binding, and peptide stability rather than comparison with a single nonmalignant cell line [2, 3, 4, 14, 15, 16, 20].

In HER2-enriched and triple-negative breast cancer models, melittin suppressed receptor phosphorylation within minutes and altered downstream AKT and mitogen-activated protein kinase (MAPK) signaling. Biophysical proximity experiments suggested that melittin acts near EGFR without behaving as a conventional competitive ligand, supporting the hypothesis that membrane remodeling interferes with receptor orientation, clustering, or dimerization [6].

Apoptosis, oxidative stress, and cell-cycle control

Mitochondrial apoptosis is the most frequently reported regulated form of cell death after exposure to bee venom or melittin. Reported changes include an increased Bcl-2-associated X protein (Bax)/ B-cell lymphoma 2 (Bcl-2) ratio, loss of mitochondrial membrane potential, cytochrome c release, activation of caspase-9 and caspase-3, and poly(ADP-ribose) polymerase (PARP) cleavage. In pancreatic cancer cells, bee venom produced dose-dependent growth inhibition, S-phase arrest, increased pro-apoptotic proteins, and reduced migration [7]. Melittin also activated caspase-dependent death and reduced clonogenic survival in leukemia and melanoma

models [9, 10].

The extrinsic apoptotic pathway contributes in selected cell models. Ovarian cancer cells showed increased death-receptor expression and caspase-8 activation together with reduced JAK2/STAT3 signaling. Silencing the death receptors weakened the antiproliferative response, supporting their functional contribution [11]. In non-small-cell lung cancer, increased death receptor 3 expression and reduced NF- κ B activity were associated with growth inhibition [12].

Oxidative stress can connect membrane injury with mitochondrial dysfunction and other forms of cell death. Calcium imbalance and respiratory-chain disruption increase reactive oxygen species, whereas depletion of glutathione-dependent defenses favors lipid peroxidation. In lung-cancer models, melittin has been associated with ferroptosis-related lipid damage and endoplasmic reticulum stress, but their contribution relative to apoptosis and direct lysis remains dependent on dose and experimental model [16, 21].

Different studies have linked melittin exposure to overlapping redox and cell-death responses. Mitochondrial reactive oxygen species can promote cardiolipin oxidation, permeability-transition events, and cytochrome c release, whereas glutathione depletion and impaired glutathione peroxidase 4 (GPX4) activity can increase phospholipid peroxidation. Endoplasmic reticulum stress may develop when calcium redistribution, oxidative protein damage, and ATP depletion impair protein folding. Protein kinase RNA-like ER kinase-Activating Transcription Factor 4-C/EBP homologous protein (PERK-ATF4-CHOP) or inositol-requiring enzyme 1-the transcription factor X-box binding protein (IRE1-XBP1) signaling may initially support adaptation and later favor apoptosis. Across different models, melittin has been associated with apoptotic, ferroptosis-related, lysosomal, and membrane-lytic effects; the contribution of each response requires pathway-specific pharmacological or genetic testing rather than inference from a single biomarker [16, 21-24].

Cell-cycle effects vary by model. Pancreatic cancer cells showed S-phase accumulation with reduced cyclins and cyclin-dependent kinases, whereas other experimental systems have reported arrest at different checkpoints depending on cell type, venom preparation, dose, and treatment combination. These differences are more consistent with a general stress response than with selective inhibition of one cell-cycle checkpoint [7, 13, 25].

Cancer-related signaling and tumor progression

Melittin exposure is frequently followed by changes in the PI3K/AKT/mTOR axis. In breast cancer and melanoma models, reduced phosphorylation of PI3K, AKT, and mTOR was associated with lower proliferation, motility, invasion, and clonogenic survival [6, 10]. EGFR or HER2 inhibition may occur upstream. Energy depletion could also contribute to mTOR suppression through AMP-activated protein kinase (AMPK), but this route has not been directly demonstrated in all cited cancer models.

MAPK responses are more variable. Extracellular signal-regulated kinase (ERK) inhibition is often associated with

reduced proliferation, whereas transient c-Jun N-terminal kinase (JNK) or p38 activation may represent a stress response that promotes apoptosis. The direction and timing of MAPK changes differ between cell lines; therefore, statements that melittin universally inhibits MAPK signaling are not supported [2, 3, 6, 10].

NF- κ B and STAT3 regulate genes involved in proliferation, resistance to apoptosis, inflammation, angiogenesis, and immune evasion. In ovarian and lung cancer models, reduced activity of these pathways accompanied death-receptor activation [11, 12]. In hepatocellular carcinoma, melittin or whole venom combined with sorafenib increased pro-apoptotic signaling and reduced NF- κ B, hypoxia-inducible factor 1 α (HIF-1 α), vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9) [13].

Antimetastatic effects include reduced migration, invasion, and matrix-metalloproteinase activity. Melanoma studies reported suppression of MMP-2 and MMP-9 together with PI3K/AKT/mTOR and MAPK changes [10]. In MDA-MB-231 breast cancer cells, melittin increased breast cancer metastasis suppressor 1 (BRMS1) and developmentally regulated GTP-binding protein 1 (DRG1) expression, whereas whole bee venom upregulated DRG1 and KAI1/CD82 and reduced WNT7B expression. These cell-based findings require validation in models of spontaneous metastasis [26].

Reduced migration and angiogenesis have been reported in several tumor models. Suppression of AKT, NF- κ B, STAT3, or hypoxia-responsive signaling can lower VEGF expression and matrix remodeling. In breast, melanoma, pancreatic, and hepatocellular models, reduced motility was associated with lower MMP-2 or MMP-9 activity or with changes in metastasis-related gene expression. Short migration assays may, however, be confounded by reduced proliferation or sublethal toxicity. Stronger evidence requires proliferation-adjusted motility assays, three-dimensional invasion, orthotopic implantation, and spontaneous-metastasis endpoints [7, 8, 10, 13, 26, 27].

Autophagy, mitophagy, and endoplasmic reticulum stress

Autophagy is a multistep lysosomal process and cannot be inferred from one marker. Microtubule-associated protein 1A/1B-light chain 3 (LC3-II) accumulation may indicate increased autophagosome formation or impaired degradation, whereas Beclin-1 upregulation identifies an initiation-related event rather than completed flux. Assessment therefore requires lysosomal inhibitors, Sequestosome 1 (p62/SQSTM1) measurements, tandem fluorescent LC3 reporters, or genetic disruption of Autophagy-related (ATG) proteins [19].

In hepatocellular carcinoma, melittin-induced autophagy behaved primarily as a cytoprotective response. Chloroquine enhanced melittin-associated apoptosis, whereas rapamycin reduced cytotoxicity, suggesting that autophagic recycling mitigated cellular damage [28]. The result provides a rationale for testing lysosomal inhibitors, but chloroquine has effects beyond autophagy and the finding requires genetic

confirmation.

Colorectal HCT116 cells exposed to melittin showed increased LC3B-II and Beclin-1, reduced p62, and activation of spliced X-box-binding protein 1 (XBP1s) and C/EBP homologous protein (CHOP), linking autophagy with the unfolded-protein response (UPR) [22]. Persistent calcium, redox, and protein-folding stress can shift the adaptive UPR toward CHOP-mediated apoptosis. *In vivo* colorectal evidence also supports an endoplasmic-reticulum-stress component in melittin-associated tumor inhibition [24].

Lung-cancer studies report different autophagic outcomes. In A549 cells, melittin damaged mitochondria and impaired terminal mitophagic clearance, allowing dysfunctional mitochondria to accumulate and generate excess reactive oxygen species [23]. In another model, melittin altered cathepsin B localization and promoted cathepsin B (CTSB)-dependent hyperautophagy; inhibition of CTSB or autophagy weakened tumor suppression *in vitro* and *in vivo* [29]. Bee venom also enhanced the effects of gefitinib in EGFR-driven lung-cancer cells while changing LC3 levels, although complete autophagic flux was not established [30].

The reviewed studies describe three distinct outcomes: cytoprotective autophagy, cytotoxic hyperautophagy, and incomplete autophagic or mitophagic flux [23, 28, 29]. Because these outcomes differ, autophagy inhibitors should not be combined with melittin without first establishing the role of autophagy in the specific tumor model [19, 22, 23, 28-30].

The intracellular site of melittin release may influence the dominant response. Free melittin initially contacts the plasma membrane, whereas liposomal or polymeric formulations may release the peptide in endosomes, lysosomes, the cytosol, or near mitochondria. Lysosomal release may increase cathepsin-dependent injury, while mitochondrial accumulation may impair mitochondrial quality control and favor apoptosis. Delivery systems should therefore be assessed for intracellular trafficking, lysosomal integrity, mitochondrial function, and timing of cell-death signals in addition to total tumor accumulation and cytotoxicity [17, 18, 23, 29-33].

A possible interpretation is that the three autophagic outcomes occur at different levels or durations of cellular stress, although this has not been demonstrated within one experimental model. At sublethal exposure, autophagy may help cells tolerate melittin-induced stress by recycling damaged proteins and organelles. With greater injury, cathepsin-dependent degradation may contribute to cell loss, whereas failure of fusion, acidification, or cargo degradation can retain damaged mitochondria and increase reactive oxygen species. The same increase in LC3-II can therefore accompany opposite biological outcomes. Functional studies should determine whether ATG5 or ATG7 silencing, lysosomal inhibition, or stimulation of flux increases or decreases melittin-induced death [19, 22, 23, 28-30].

Combination therapy and immune modulation

Combination studies have tested whether melittin can increase sensitivity to established anticancer agents. Melittin

enhanced docetaxel activity in breast-cancer models [6], whereas bee venom or melittin increased sorafenib-associated apoptosis in hepatocellular carcinoma models [13]. In EGFR-dependent lung-cancer cells, bee venom also increased the antiproliferative and antimigratory effects of gefitinib [30]. Bee venom combined with tamoxifen, hesperidin, and piperine changed Bax, caspase-3, Bcl-2, and VEGF in a breast-cancer animal model, but the multi-agent design prevents attribution of the result to one component [27].

Recent studies have evaluated combinations with thymoquinone, demonstrating enhanced apoptosis and cell-cycle disruption *in vitro* [25]. Egyptian bee venom also inhibited sensitive and resistant breast-cancer cells and reduced Ehrlich tumor growth in mice, but the model does not reproduce the molecular heterogeneity of human breast cancer [34].

Melittin-induced membrane injury can release tumor antigens and danger signals, creating a rationale for immunotherapy. Polymeric D-melittin nanoparticles and other formulations have been designed to produce immunogenic cell death while reducing direct hemolysis [18, 33]. In a subcutaneous KPC pancreatic tumor model in mice, peritumoral crude bee venom combined with intraperitoneal anti-PD-L1 blockade and oral epacadostat, an indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, was associated with improved tumor control and changes in CD4-positive and CD8-positive T-cell populations and activation markers at the tumor site and in the spleen. However, CD69 and CD38 responses were not uniform across cell populations or treatment groups. These findings remain preliminary: the experiments used a single subcutaneous tumor model, small groups of five to ten mice, short follow-up, and limited immune phenotyping; they do not establish clinical efficacy or durable antitumor immunity [35].

Evidence of immunogenic cell death requires more than membrane rupture. Relevant measurements include calreticulin exposure, extracellular ATP, High Mobility Group Box 1 (HMGB1) release, dendritic-cell activation, and tumor-specific T-cell responses. D-melittin nanoparticles have induced several of these signals in animal models, but durable immunity should be confirmed by tumor rechallenge, memory T-cell analysis, and control of distant lesions. Uncontrolled venom exposure may instead activate mast cells, complement, or inflammatory cytokines without producing tumor-specific immunity [18, 33, 35].

Targeted delivery and nanomedicine

Systemic administration of free melittin is limited by rapid interaction with erythrocytes, endothelium, plasma proteins, and immune components. Investigated delivery systems include lipid-coated polymeric nanoparticles, liposomes, niosomes, micelles, tumor-cell-membrane-coated particles, inorganic nanomaterials, antibody-directed carriers, and constructs activated by acidic pH or tumor proteases [14-18, 31-33].

Nanocarriers can reduce direct contact between melittin and erythrocytes, modify circulation time, increase tumor

accumulation, and control intracellular release. Polymeric D-melittin formulations have shown antitumor activity with reduced systemic toxicity in animal models [33]. Molecularly targeted perfluorocarbon carriers delivered melittin to tumors in mice without immediate generalized lysis [36].

Carrier design affects both biodistribution and the intracellular site of release. Plasma-membrane release may favor pore formation, whereas endosomal or lysosomal release may alter autophagy and cathepsin-dependent cell death. Local concentration also influences whether the predominant outcome is signaling change, apoptosis, lytic death, or inflammatory tissue injury [18, 20, 31].

A useful carrier must prevent premature peptide leakage and erythrocyte exposure, remain stable in plasma, reach tumor tissue, penetrate beyond perivascular regions, and release active melittin at the intended site. Longer circulation may improve tumor exposure but may also increase complement activation and hepatic or splenic uptake. Biomimetic coatings, receptor-targeting ligands, protease-cleavable masks, pH-responsive materials, and D-amino-acid analogues address different limitations. Direct comparisons under standardized conditions are still uncommon [17, 18, 31, 32, 33, 36].

No published cancer clinical study identified in this review had established the safety or antitumor efficacy of a melittin-based nanoparticle. Manufacturing reproducibility, premature peptide release, complement activation, hepatic and splenic uptake, repeat-dose immunogenicity, and carrier biodegradation remain unresolved [14, 17, 18, 31-33].

Clinical evidence and safety

Clinical publications on bee venom in oncology are sparse and should not be conflated with preclinical antitumor evidence. A clinical literature review identified one randomized study focused on cancer-related pain, several case series addressing chemotherapy-induced peripheral neuropathy, and case reports involving heterogeneous malignancies. These studies primarily evaluated symptoms and quality of life rather than objective tumor response or survival [37].

Bee venom acupuncture or pharmacopuncture may expose patients to local pain, erythema, edema, infection risk, and systemic hypersensitivity. Whole venom contains major allergens, including PLA2 and hyaluronidase, and can provoke bronchospasm or anaphylaxis. Melittin contributes to pain and hemolysis. The narrow and incompletely characterized therapeutic window is therefore a central translational barrier [3, 38-39].

Current evidence does not support replacing surgery, radiotherapy, chemotherapy, targeted therapy, or immunotherapy with bee venom, bee stings, or free melittin. A clinically credible development program would require a standardized purified or engineered product, validated tumor targeting, pharmacokinetic and biodistribution studies, hemolysis and immunotoxicity testing, and phased clinical evaluation [2, 3, 37-39].

Translation would require a staged development pathway similar to that used for other cytotoxic peptides. The investigational product must first be chemically defined

and manufactured reproducibly, followed by validated assays for potency, aggregation, residual solvent, endotoxin, peptide leakage, and storage stability. Nonclinical studies should establish hemolytic thresholds, complement activation, cytokine release, cardiovascular and renal toxicity, reproductive toxicity where relevant, biodistribution, and repeated-dose immunogenicity. Only after a delivery system demonstrates a convincing exposure-safety relationship would phase I oncology trials be justified. Such trials would primarily assess safety, pharmacokinetics, and pharmacodynamic target engagement, not assume clinical antitumor efficacy from cell-culture potency [3, 14, 17, 18, 31-33, 36-39].

Discussion

Across the reviewed studies, membrane disruption is the most consistently documented effect of melittin, whereas evidence for direct intracellular targets varies by model. Stronger support for a causal pathway comes from rapid receptor changes, pharmacological rescue, genetic silencing, direct binding studies, or in vivo confirmation. Phosphorylation changes measured after extensive cytolysis should not be interpreted as selective target engagement [2, 3, 6, 19, 20].

Whole venom and purified components should not be treated as equivalent. Interactions among melittin, PLA2, and minor peptides may increase cytotoxicity in some systems, but whole venom is less reproducible and more allergenic. Purified melittin can be chemically standardized but remains hemolytic. Engineered analogues and delivery systems may improve control of exposure, although carrier composition, release kinetics, and intracellular trafficking can change the cellular response [1-5, 14, 15, 17, 18].

No single pathway explains the response across tumor types. EGFR/HER2 interference is prominent in selected breast-cancer models, JAK2/STAT3 and death receptors contribute in ovarian cancer, and autophagy can either reduce or increase melittin-induced cell death. These differences are consistent with variation in tumor genotype, membrane composition, basal metabolic stress, lysosomal capacity, and exposure conditions [6, 11, 19, 22, 23, 28, 29].

The main translational limitation is the difference between direct cell-culture exposure and clinically achievable systemic exposure. Intravenously administered melittin encounters plasma proteins, erythrocytes, endothelium, liver, spleen, and immune surveillance before reaching a tumor. Intratumoral administration improves local exposure but is not applicable to most disseminated disease. Orthotopic, metastatic, and immunocompetent models are therefore more informative than subcutaneous xenografts alone [14, 17, 18, 31-33, 36].

Additional methodological weaknesses include insufficient chemical characterization of venom batches, limited use of matched normal cells, reliance on two-dimensional monolayers, variable exposure durations, incomplete autophagic-flux assessment, and publication bias toward positive findings. Comparative studies should report melittin

and PLA2 content, endotoxin status, hemolytic indices, plasma stability, and the relationship between cytotoxic and non-cytolytic concentrations [2, 3, 14, 15, 19].

Future studies should test potential predictors of response rather than assume uniform tumor sensitivity. Candidate variables include phosphatidylserine exposure, EGFR or HER2 dependence, cholesterol and glycosphingolipid organization, CTSB localization, basal autophagic flux, and antioxidant capacity. Organoids, patient-derived tumor models, microphysiological systems, and spatial analysis of tumor-immune interactions can determine whether apparent selectivity persists in complex tissues [2, 3, 6, 19, 22, 23, 29].

Bee venom should be treated as a source of candidate drug structures, not as an alternative oncology treatment. Unstandardized venom administration is not equivalent to development of a regulated pharmaceutical product, and this distinction is essential for patient safety [2, 3, 14, 37-39].

For the Republic of Moldova, an initial research program could include chemical profiling of locally collected venom, quantification of melittin and PLA2, testing in matched tumor and normal-cell models, and standardized hemolysis assays. Collaboration among apiculture specialists, biochemists, oncologists, toxicologists, and materials scientists could assess geographic and seasonal variation and develop reproducible formulations. The immediate goals should be target identification, validation of cellular pathways, and preclinical safety rather than clinical use [1, 2, 4, 5, 15].

Evidence strength differs by endpoint. Membrane disruption and concentration-dependent cytotoxicity are consistently documented in model membranes and cell systems, whereas tumor-selective systemic exposure has not been demonstrated. Selected studies support apoptosis through caspase activation, mitochondrial depolarization, and rescue experiments, but these events may still follow membrane injury. Evidence for reduced migration, angiogenesis, and immune evasion is less robust because few studies use orthotopic tumors, spontaneous metastasis, or longitudinal immune analyses. Nanocarriers improve delivery in animal models but do not constitute clinical evidence of efficacy [3, 14, 17, 18, 20, 21, 31-33, 36, 38, 39].

Future comparative experiments should use reference standards for melittin purity and venom composition, report free and carrier-bound peptide concentrations, and distinguish nominal dose from biologically available exposure. A core assay set could include real-time membrane permeability, erythrocyte hemolysis, endothelial viability, mitochondrial respiration, caspase dependence, autophagic flux, cytokine release, and clonogenic recovery. *In vivo* studies should compare intravenous, intratumoral, and targeted formulations with equivalent peptide exposure and should include pharmacokinetic sampling, organ histology, complement activation, and metastatic endpoints. Such harmonization would allow researchers to determine whether a new formulation genuinely widens the therapeutic index or merely shifts toxicity to a different tissue or time point [14, 17-19, 31-33, 36].

Clinical development depends on whether a defined formulation can maintain therapeutically relevant intratumoral exposure without exceeding safety limits for hemolysis, endothelial injury, complement activation, and anaphylaxis. Study design and reporting should focus on this exposure-safety relationship rather than equating strong *in vitro* cytotoxicity with a clinically usable therapeutic window [3, 14, 17, 18, 31, 36-39].

The Moldovan context supports local research but does not provide direct evidence for bee-venom therapy. Cancer mortality has shown an unfavorable long-term pattern in the Republic of Moldova, including reported increases in lung, breast, and intestinal cancer mortality after the beginning of the millennium [40]. A small Moldovan survey of young adults reported awareness and use of beekeeping products, illustrating cultural familiarity with apiculture-derived products but not anticancer efficacy [41]. Moldovan researchers have also provided a broader biochemical framework for studying oxidative stress, antioxidant activity, and bioactive natural products; this work supports national research capacity but is not direct evidence for the anticancer activity of bee venom or melittin [42]. Melittin production in honeybees varies with age, season, and physiological condition. Experimental studies also suggest that *Varroa destructor* infestation can impair melittin production. *Varroa* infestation is associated both with increased oxidative stress and with reduced melittin production, that is why venom intended for research should be obtained from characterised, healthy colonies; the cited studies do not, however, establish a direct causal relationship between hemolymph antioxidant capacity and melittin biosynthesis [43-45].

A perspective is particularly appropriate because bee-venom research links human oncology with pollinator health, biodiversity, apiculture, environmental quality, and biotechnology. Sustainable research should use standardized nonlethal collection, traceable samples, contaminant monitoring, and practices that protect colony health. Pharmaceutical development should not encourage uncontrolled stinging practices or extraction methods that harm bees or beekeeping systems [43-47].

Conclusions

Preclinical studies associate bee venom and melittin with membrane disruption, altered growth-factor receptor signaling, mitochondrial and death-receptor-mediated apoptosis, changes in PI3K/AKT/mTOR, MAPK, janus kinase/signal transducer and activator of transcription (JAK/STAT), and NF- κ B pathways, altered autophagy and organelle stress, cell-cycle arrest, and reduced invasion or angiogenesis.

The response depends on concentration, tumor type, exposure duration, venom composition, and intracellular delivery. Autophagy can reduce cell damage, contribute to cell death, or remain incomplete; its role must therefore be established separately in each tumor model.

The main barriers are hemolysis, allergenicity, injury to

normal membranes, variability of whole-venom preparations, uncertain pharmacokinetics, and the absence of convincing clinical antitumor evidence. Targeted nanocarriers and engineered melittin analogues are actively investigated, but they still require standardized comparative toxicology

and controlled clinical evaluation. Bee venom and melittin represent sources of bioactive compounds for anticancer drug development. However, their therapeutic potential remains preclinical, and current evidence does not support their use as alternatives to established cancer treatments.

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