



The therapeutic potential of bee venom-derived Apamin and Melittin conjugates in cancer treatment: A systematic review

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ABSTRACT

The therapeutic potential of bee venom-derived peptides, particularly apamin and melittin, in cancer treatment has garnered significant attention as a promising avenue for advancing oncology. This systematic review examines preclinical studies highlighting the emerging role of these peptides in enhancing cancer therapies. Melittin and apamin, when conjugated with other therapeutic agents or formulated into novel delivery systems, have demonstrated improved efficacy in targeting tumor cells. Key findings indicate that melittin-based conjugates, such as polyethylene glycol (PEG)ylated versions, show potential in enhancing therapeutic outcomes and minimizing toxicity across various cancer models. Similarly, apamin-conjugated formulations have improved the efficacy of established anti-cancer drugs, contributing to enhanced targeting and reduced systemic toxicity. These developments underscore a growing interest in leveraging bee venom-derived peptides as adjuncts in cancer therapy. The integration of these peptides into treatment regimens offers a promising strategy to address current limitations in cancer treatment, such as drug resistance and off-target effects. However, comprehensive validation through clinical trials is essential to confirm their safety and effectiveness in human patients. This review highlights the global emergence of bee venom-derived peptides in cancer treatment, advocating for continued research and development to fully realize their therapeutic potential.

1. Introduction

Cancer continues to be a major global health challenge [1–3], characterized by its heterogeneity [4–6] and resistance to conventional treatments such as chemotherapy [7] and radiation therapy [8]. Despite

significant advancements in these areas, the search for more effective and targeted therapies remains crucial [9,10]. Novel approaches are needed to overcome the limitations of existing treatments and to provide personalized care that addresses the specific characteristics of each tumor [11–13]. In recent years, there has been growing interest in the

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use of natural products for cancer therapy [14–16]. Bee venom, a complex mixture of biologically active compounds, has emerged as a particularly intriguing source of potential therapeutic agents [17]. Among its constituents, apamin [18] and melittin [19] have garnered attention for their distinct mechanisms of action and promising pre-clinical results.

Apamin (Fig. 1) is a small peptide that belongs to the neurotoxin family found in bee venom [20]. It is known for its ability to selectively inhibit calcium-dependent potassium channels [21], which can lead to various biological effects, including modulation of inflammatory responses [22] and induction of cell apoptosis [23]. In the context of cancer, apamin has been explored for its potential to selectively target and kill cancer cells, particularly when conjugated with other therapeutic agents or delivery systems [24,25]. Melittin (Fig. 2), another key component of bee venom, is a 26-amino-acid peptide with potent membrane-disrupting properties [26]. It has been shown to induce apoptosis in cancer cells by creating pores in the cell membrane, leading to cell lysis [27]. Melittin's ability to target and disrupt lipid membranes arises from the oligomerization of melittin peptides to form trans-membrane channels. Indeed, melittin's ability to cause cell lysis through pore formation emerges from the molecule's ability to directly target the membrane lipids rather than a specific protein. These channels lead to osmotic cell lysis. From a molecular perspective, each melittin peptide has a two α -helical segment arrangement. These two α -helical segments are joined to form a "bend rod" configuration due to disruption of the helical structure, which happens at amino acid positions 10–12 [28]. Once melittin binds to the cells' lipid membranes, toroid-shaped pores are formed. These pores enable the leakage of cellular molecules with sizes ranging tens of kDa. This peptide's actions change the cell membrane's permeability [29]. Unfortunately, the resolution of these pores' specific atomic structure is still unknown [30]. Many studies' results confirm membrane pore formation due to melittin's effects but are inconsistent regarding the insertion-to-toroidal pore pathway [31]. Additionally, melittin has demonstrated anti-inflammatory [32] and

anti-angiogenic [33] effects, making it a versatile candidate for cancer therapy. Recent research has focused on enhancing the stability and targeting of melittin through various nanocarriers and conjugation strategies [34–36].

Melittin causes apoptosis of cancerous cells by both the mitochondrial and death receptor (DR) pathways. As an example of melittin's ability to cause apoptosis via the mitochondrial pathway, melittin was found to kill alveolar basal epithelial adenocarcinoma cells (A549) through the B-cell lymphoma protein 2 (Bcl-2) associated X (Bax)/Bcl-2 mitochondria-related apoptosis pathway by targeting mitochondria directly [37]. Melittin also induces gastric cancer cell apoptosis via the mitochondrial pathway, which ultimately increases the release of cytochrome C and endonuclease G and exacerbates caspase (CAS) activity [38]. On the other hand, melittin was found to cause ovarian cancer cell death via inhibition of the Janus kinase/signal transducers and activators of transcription (JAK2/STAT3) pathway and induction of DR activation. In these cells, expression of the DR3 and DR6 was slightly increased [39]. Different from melittin, apamin-induced cancer cell apoptosis may occur mainly via the mitochondrial pathway in combination with invasomes (INVA). For example, optimized 2-methoxyestradiol (2ME) INVA apamin-fortified induced suppression of lung A549 cancer cells via the mitochondrial pathway. This effect is indicated due to reduced Bcl-2 expression and increased Bax expression following the complex administration. The complex also causes loss of cancer cell mitochondrial membrane potential [18]. Apamin conjugates can induce apoptosis through DR pathways, possibly via the tumor necrosis factor alpha (TNF- α) receptor pathway. Additionally, apamin conjugates can regulate nuclear factor kappa B (NF- κ B) and, therefore, apoptosis via other DR [24,25]. Unfortunately, apamin's potential to mitigate cancer cell survival through apoptosis via DR pathways has not yet been tirelessly studied.

The development of novel nanoformulation technologies has significantly advanced the application of these bee venom-derived peptides in cancer treatment. These technologies enhance the bioavailability [42],

Apamin

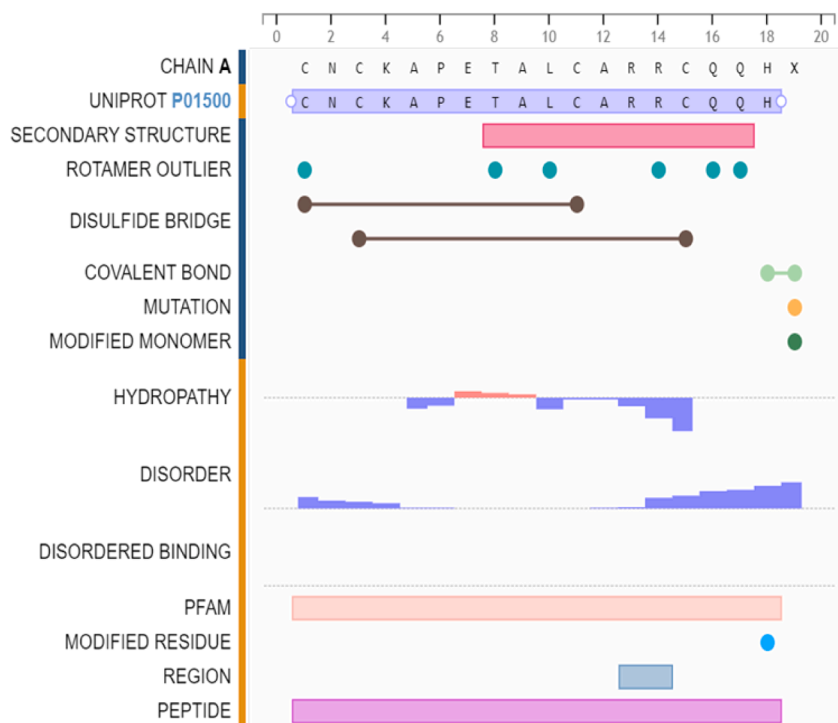
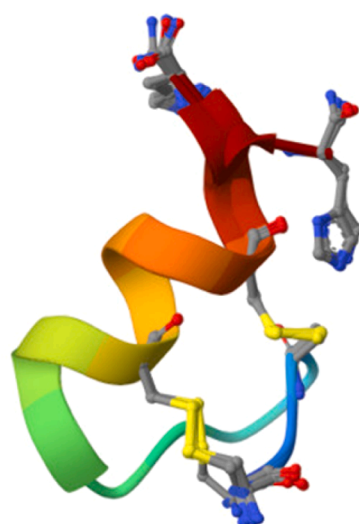


Fig. 1. The figure displays the molecular structure of apamin, which has a molecular formula of $C_{79}H_{131}N_3O_{24}S_4$ and a molecular weight of 2027.4 g/mol. The compound features an XLogP₃-AA of −13.4, with 31 hydrogen bond donors and 33 hydrogen bond acceptors. It has 37 rotatable bonds, an exact mass of 2025.8866089 g/mol, and a topological polar surface area of 1020 Å². Apamin's heavy atom count is 138 and carries no formal charge. Its complexity is rated at 4320, with 19 defined atom stereocenters. The structure is canonicalized [40].

Melittin

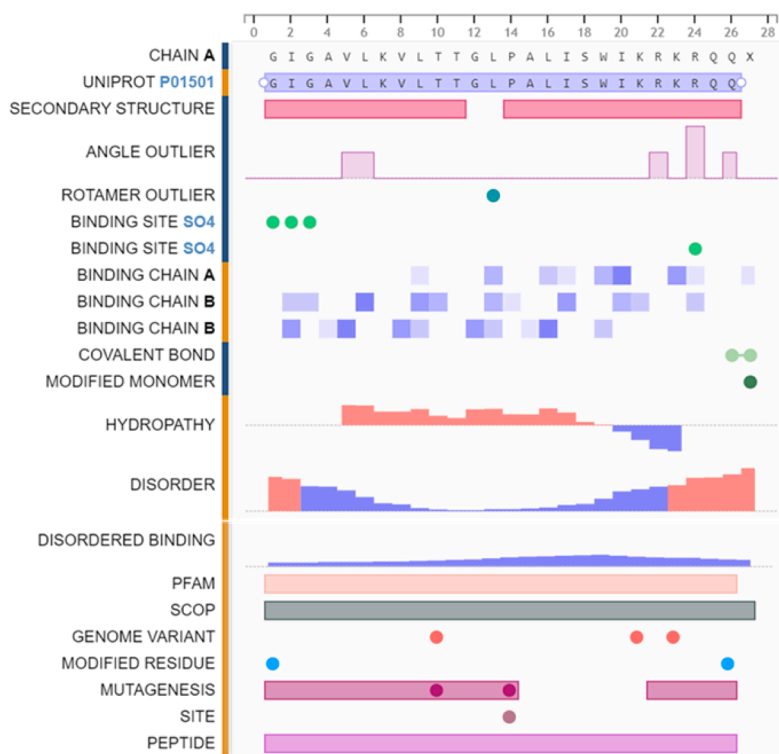


Fig. 2. This figure shows the molecular structure of melittin, a peptide with 20 hydrophobic N-terminal residues and 6 basic C-terminal residues, making it water-soluble. Melittin is moderately soluble in methanol at 27°C and exhibits unique unfolding properties in aqueous and methanol solutions. When embedded in lipid bilayers, melittin retains its helical structure and can form dimers that destabilize artificial lipid bilayers, causing membrane leakage. In its monomeric form, it has minimal effect on lipid bilayers. Identified through X-ray crystallography, NMR, CD, and Raman spectroscopy, melittin features m/z values for its charged ions: 949.8 ($[M + 3 H]^3+$), 712.5 ($[M + 4 H]^4+$), 570.2 ($[M + 5 H]^5+$), and 475.3 ($[M + 6 H]^6+$), with molecular weights of 2846 Da (melittin), 2868 Da (melittin + Na⁺), and 2884 Da (melittin + K⁺). Two isoforms are noted: melittin-S, with a ¹⁰Ser substitution, and melittin-F, a fragment missing seven N-terminal residues [41].

stability [43], and targeted delivery [44] of apamin and melittin, potentially improving their therapeutic efficacy [45] while minimizing off-target effects [46] and toxicity [47].

This systematic review is unique as it specifically addresses the role of bee venom-derived apamin and melittin conjugates in cancer treatment. To date, no systematic review has focused exclusively on these peptides and their conjugates in the context of cancer therapy. By synthesizing and analyzing preclinical evidence from studies involving apamin and melittin, particularly those utilizing advanced nano-formulation techniques, this review aims to provide a detailed overview of their potential as therapeutic agents. Our review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines to ensure a rigorous and transparent literature examination. We highlight key findings from the studies, including these bee venom-derived compounds' mechanisms of action, efficacy, and safety profiles. Additionally, we identify gaps in the current research and propose directions for future studies to facilitate the translation of these findings into clinical applications. Ultimately, this review seeks to offer a comprehensive assessment of the therapeutic potential of apamin and melittin, contributing to the ongoing efforts to develop more effective and targeted cancer treatments.

2. Literature search methodology

2.1. Database search

A comprehensive literature search was conducted using PubMed, Scopus, and Web of Science. Keywords such as "melittin," "apamin," "cancer therapy," "preclinical studies," "therapeutic efficacy," and "nanocarriers" were employed to identify relevant studies. Boolean operators like AND, OR, and NOT were utilized to refine the search results;

for example, "melittin AND cancer therapy" or "apamin OR melittin NOT review." Filters such as publication date (last decade), language (English), and document type (original research articles) were applied to ensure the relevance and quality of the studies included. The search strategy aimed to capture a broad range of studies focusing on melittin- and apamin-based therapies in cancer treatment, ensuring a comprehensive review of the available evidence. This approach allowed us to gather a diverse and extensive collection of research articles, providing a robust foundation for analyzing the preclinical efficacy and potential therapeutic applications of bee venom-derived apamin and melittin conjugates in cancer therapy.

2.2. Inclusion criteria

Studies were selected based on several criteria. They had to be published in peer-reviewed journals and focus on preclinical evaluations (either in vitro or in animal models) of bee venom-derived apamin and melittin conjugates for cancer therapy due to the absence of clinical trials. We included studies that provided detailed information on the intervention, control, endpoints, results, and conclusions. Only articles published in the last decade were considered to ensure the relevance of the findings to current research and advancements. Additionally, only studies published in English were included to maintain consistency and ensure accurate interpretation of the data.

2.3. Exclusion criteria

Studies were excluded if they focused solely on reviews or were not available in English. Articles that lacked full text or did not meet the preclinical study criteria were omitted. This exclusion process was essential to ensure that only relevant and accessible research was

included in the review, thereby maintaining a high standard of evidence and facilitating accurate analysis and interpretation of the data.

2.4. Study selection

Following the initial search, articles were screened for relevance. Full-text articles were reviewed to extract detailed information on objectives, models, interventions, controls, endpoints, results, conclusions, possible clinical implications, and study limitations. This structured approach allowed for a thorough assessment and categorization of the studies into two tables based on the peptide used: melittin-based and apamin-based therapies.

2.5. Quality assessment

To assess the quality of the studies included in our review, a systematic approach was employed. Initially, the methodological rigor of each study was evaluated based on several key factors: the design and execution of experiments, the appropriateness of models and controls, and the clarity in defining interventions and endpoints. Each study's sample size was examined to ensure it was adequate for drawing reliable conclusions, and the comprehensiveness of data reporting was assessed to verify that results were presented with sufficient detail. Additionally, potential biases were identified, including variability in experimental conditions and short-term versus long-term outcomes. Special attention was given to the studies' ability to translate preclinical findings into potential clinical implications, recognizing that thorough validation in animal models and subsequent clinical trials is crucial. By systematically

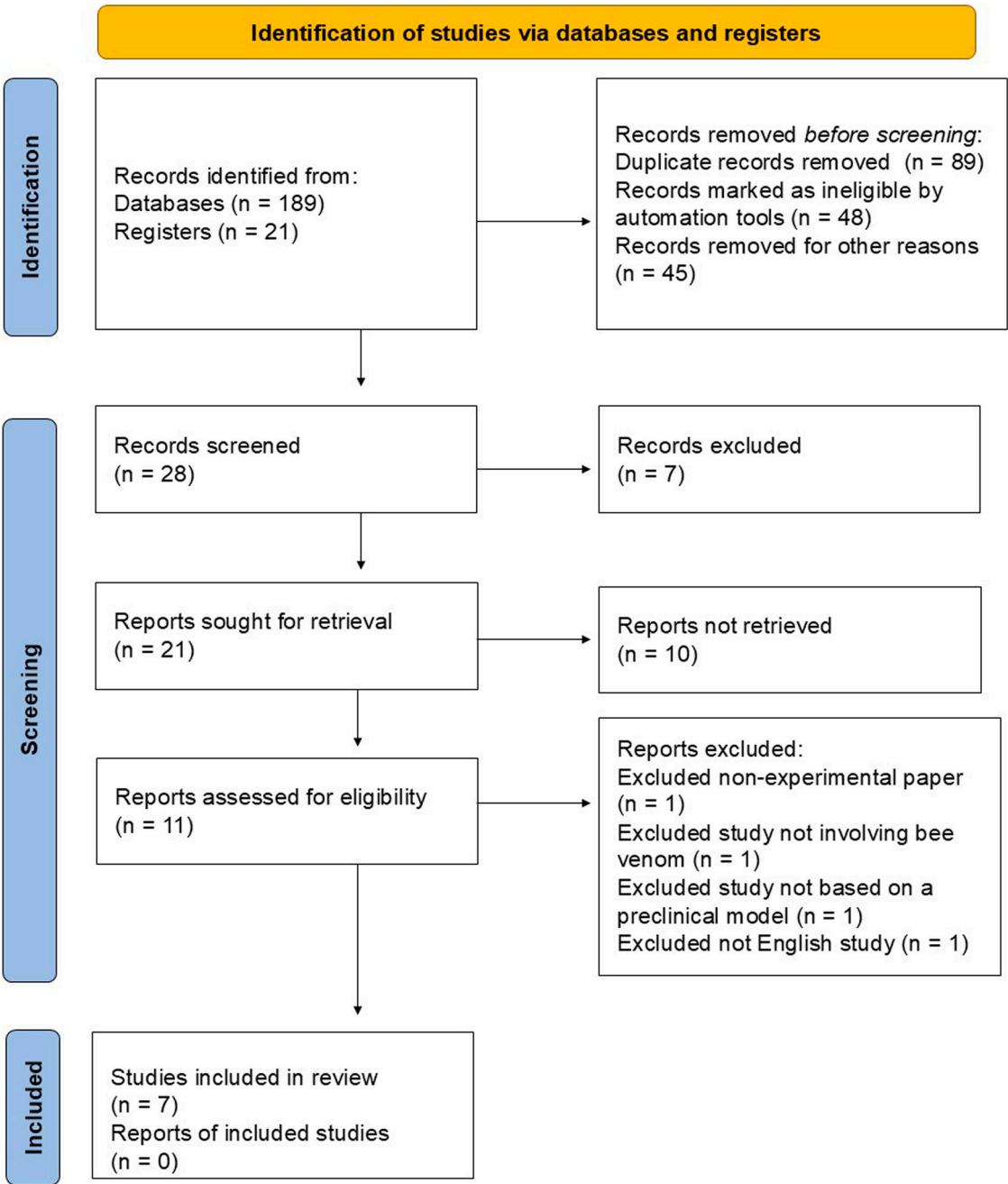


Fig. 3. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [48] Diagram of Study Selection Process.

applying these criteria, we aimed to ensure a thorough and objective evaluation of the studies' overall quality and relevance to advancing melittin- and apamin-based cancer therapies.

3. Literature search PRISMA findings and quality overview of included studies

To provide a clear overview of the study selection process for this review, the PRISMA [48] diagram below (Fig. 3) illustrates the flow of information through the various stages, from initial identification of studies to final inclusion in the systematic review. Records were identified from databases ($n = 189$) and registers ($n = 21$). Before screening, duplicate records ($n = 89$) were removed, and records marked as ineligible by automation tools ($n = 48$) were excluded, along with records removed for other reasons ($n = 45$). This left a total of 28 records for screening. Out of these, 7 records were excluded. Reports were then sought for retrieval, totaling 21. However, 10 reports were not retrieved. Following this, 11 reports were assessed for eligibility. Four reports were excluded based on specific criteria: one non-experimental paper, one study not involving bee venom, one study not based on a preclinical model, and one study not in English. Finally, 7 studies were included in the review. These studies covered various cancers, including melanoma, cervical cancer, lung cancer, pancreatic cancer, and breast cancer.

Jeong et al. [49] employed a comprehensive approach using a mouse model, with multiple endpoints such as tumor growth, survival rates, and immune cell profiles. This study is methodologically strong due to its use of appropriate controls and a variety of measurements. However, the limitations include potential variability in tumor models, which can affect the generalizability of the results, and the absence of long-term safety data in humans, which is crucial for assessing the full therapeutic potential and risks of the melittin-mertansine (DM1) conjugate (M-DM1). The possible clinical implications suggest that this research could lead to improved therapies for melanoma by targeting specific tumor-associated macrophages (TAMs), potentially enhancing patient survival and providing a more targeted approach to treatment.

Kim et al. [35] employed a detailed comparative approach using a mouse model to assess a new melittin-dKLA formulation. The study focused on relevant endpoints, including tumor growth and metastasis, and is methodologically sound with clear comparisons. However, limitations include unspecified controls, which could affect the reliability of the results, and the need for further investigation into long-term safety and broader applicability. The formulation's effectiveness in reducing tumor growth and metastasis is promising, but its safety profile and efficacy across different cancer types need more exploration. The possible clinical implications suggest that this formulation could provide a safer and more effective treatment for triple-negative breast cancer (TNBC), with reduced side effects, potentially improving patient outcomes and offering a new therapeutic option for a challenging cancer type.

Jia et al. [34] employed a comprehensive method in evaluating a polyethylene glycol (PEG)ylated melittin conjugate, focusing on pharmacokinetics and safety in a mouse model of lung cancer. The study is methodologically robust, with thorough assessments of drug delivery and toxicity. Limitations include potential issues with clinical scale-up and formulation stability, which are critical for translating preclinical successes into clinical practice. The formulation showed promising improvements in safety and efficacy, but these findings need to be confirmed in larger, more diverse patient populations. The possible clinical implications suggest that this formulation could enhance the safety and efficacy of melittin-based therapies, improve drug delivery, and reduce side effects, potentially leading to better patient adherence and treatment outcomes in clinical settings.

Sahsuvar et al. [36] employed an in vitro approach using multiple cancer cell lines to assess targeted and redox-responsive melittin conjugates. The study is of moderate to high quality due to its comprehensive evaluation of anticancer efficacy and additional properties such

as antioxidant effects. However, the lack of in vivo validation limits the applicability of the findings, as in vitro results may not fully represent the complex interactions in a living organism. The possible clinical implications suggest that these conjugates could offer more effective and less toxic treatments for cervical cancer, with additional therapeutic benefits.

Awan et al. [18] employed an in vitro approach to enhance 2ME's effectiveness using apamin. The study is of good quality, with a detailed analysis of drug efficacy and mechanisms of action. Limitations include the challenge of translating in vitro results to clinical application, as well as the lack of long-term safety data, which is essential for evaluating the full therapeutic potential and risks of the combination. The possible clinical implications suggest that this approach could lead to more effective treatments for lung cancer with reduced doses and side effects.

Alhakamy et al. [24] employed an in vitro approach to develop apamin and alendronate sodium nanocomplexes for pancreatic cancer therapy. The study is methodologically strong due to its thorough evaluation of cytotoxicity and apoptotic effects. However, limitations include the absence of in vivo validation, which is necessary to confirm the effectiveness and safety of the nanocomplexes in a living organism, and potential unknown long-term safety issues. The possible clinical implications suggest that these nanocomplexes could improve pancreatic cancer treatment outcomes with a more targeted and less toxic approach.

Badr-Eldin et al. [25] employed an in vitro approach to investigate apamin-functionalized emulsomes to enhance ellagic acid's cytotoxic activity against breast cancer. The study demonstrates high quality with detailed endpoints related to cytotoxicity and apoptosis. Limitations include the lack of in vivo efficacy data and the need for further optimization in clinical settings. These factors are critical for ensuring that the formulation is both effective and safe in humans. The possible clinical implications suggest that this formulation could improve breast cancer treatment efficacy and reduce systemic side effects.

4. Exploring the therapeutic potential of bee venom-derived apamin and melittin conjugates: future research directions and opportunities in cancer treatment

Tables 1 and 2 provide a comprehensive summary of preclinical studies evaluating melittin- and apamin-based therapies for cancer treatment. Table 1 details various studies investigating melittin-based interventions, including the effects of M-DM1 on TAMs and survival rates in melanoma models, the enhanced therapeutic potential of PEG-melittin-dKLA 8–26 in TNBC, and the improved pharmacokinetics and tumor suppression of polymer-assisted compaction of melittin (pac-MEL), a PEGylated melittin conjugate, compared to free melittin. It also covers research on redox-responsive melittin conjugates and their anticancer efficacy, along with their antioxidant and antibacterial benefits. Table 2 summarizes studies on apamin-based therapies, highlighting innovations such as apamin-enhanced 2ME formulations, apamin-alendronate sodium nanocomplexes for pancreatic cancer, and apamin-functionalized emulsomes to boost ellagic acid's cytotoxicity against breast cancer. Each table provides a detailed overview of study objectives, methodologies, results, and potential future research directions. Below, readers will find a detailed explanation of each study alongside future research endeavors based on these research findings. These sections will provide a comprehensive look at the current advancements, as well as outline promising directions for future research to optimize and expand the application of bee venom-derived peptide conjugates in cancer treatment.

Jeong et al. [49] demonstrated that the M-DM1 notably reduced M2-like TAMs, suppressed tumor growth, and improved survival rates in a mouse model of melanoma. M-DM1's ability to increase CD8⁺ T cells and natural killers (NK) cells further underscores its potential for enhancing immune responses against tumors. Future research could delve deeper into understanding the mechanistic pathways through

Table 1
Summary of Preclinical Studies on Melittin-Based Therapies for Cancer Treatment.

References	Objective	Model	Intervention	Endpoints	Results	Conclusion	Possible Clinical Implications	Study's Limitations
Jeong et al. [49]	Evaluate the effects of M-DM1 on M2-like TAMs and tumor progression	Mouse model; Melanoma	M-DM1 20 nmol/kg every 3 days for 15 days	Tumor growth, survival rates, TAMs phenotypes, leukocyte infiltration, expression profiles	M-DM1 reduced M2-like TAMs, suppressed tumor growth, improved mouse survival, increased CD8 ⁺ T cells and NK cells	M-DM1 shows enhanced anti-tumor effects	Could lead to improved therapies for melanoma by targeting specific TAMs; may enhance patient survival	Potential variability in tumor models; long-term effects and safety in humans not yet assessed
Kim et al. [35]	Improve therapeutic anticancer potential of MEL-dKLA by reducing toxicity and increasing stability	Mouse model; TNBC	MEL-dKLA or PEG-MEL-dKLA 8–26 200 nmol/kg administered intraperitoneally every 3 days, with a total of eight injections	Tumor growth, survival rates, lung metastasis	PEG-MEL-dKLA 8–26 showed superior effects on tumor growth, survival rates, and lung metastasis compared to MEL-dKLA	PEG-MEL-dKLA 8–26 is a potential new drug for TNBC and metastasis targeting TAMs	Could provide a safer and more effective treatment for TNBC; may reduce side effects and improve patient outcomes	Specifics of long-term safety and clinical efficacy need further investigation; generalizability to other cancer types needs to be evaluated
Jia et al. [34]	Develop pacMEL to improve safety, pharmacokinetics, and tumor targeting	Mouse model; NSCLC	MEL or pacMEL 3 mg/kg (MEL-basis) every 3 days for 30 days	Tumor accumulation, pharmacokinetics, hemolytic activity, hepatic damage, immune response	pacMEL showed ~10-fold increase in AUC, reduced hepatic damage and hemolytic activity, enhanced tumor suppression	pacMEL is a safer and more effective MEL-based drug candidate	Potential for improved safety and efficacy of MEL-based therapies; may enhance drug delivery and reduce side effects	Clinical trials needed to confirm efficacy and safety; potential issues with scale-up and formulation stability
Sahsuvar et al. [36]	Design and test targeted and redox-responsive MEL conjugates for improved cervical cancer therapy	In vitro (MCF-7, C33A, HeLa)	CM-Target or CM-FA 8–256 µg/mL across different cancer cell lines, measured over 24 hours	Anticancer efficacy, antioxidant and antibacterial effects, hemolytic activity	Both conjugates showed effective anticancer activity, reduced hemolytic activity, and had additional antioxidant and antibacterial effects	Targeted and redox-responsive MEL conjugates may offer fewer adverse effects and improved cancer treatment	Could offer more effective and less toxic treatments for cervical cancer; may also provide additional therapeutic benefits through antioxidant and antibacterial properties	In vitro findings need to be validated in animal models and clinical trials; potential for limited specificity or off-target effects

Abbreviations: AUC, Area Under the Curve; C33A, HPV (–) Human Cervical Squamous Carcinoma; CM-FA, N-terminus Cysteine Modified-Melittin–Folic Acid Functionalized Conjugate; CM-Target, N-terminus Cysteine Modified-Melittin–Targeting Peptide-functionalized Conjugate; DM1, Mertansine; HeLa, HPV (+) Human Cervical Adenocarcinoma; MCF-7, Human Breast Cancer; M-DM1, Melittin-DM1 conjugate; MEL, Melittin; NK, Natural Killer; NSCLC, Non-small Cell Lung Cancer; pacMEL, Polymer-assisted Compaction of Melittin; PEG, Polyethylene Glycol; TAMs, Tumor-Associated Macrophages; TNBC, Triple-negative Breast Cancer.

which M-DM1 influences TAMs polarization and the overall tumor microenvironment. This includes exploring how M-DM1 might interact with other immune cell types and signaling pathways involved in cancer progression. Additionally, examining the effects of M-DM1 on different cancer types with varying TAMs profiles could reveal its broader applicability. Investigating the impact of M-DM1 on cancer metastasis and its potential role in preventing tumor spread could provide valuable insights into its comprehensive anti-cancer capabilities.

Kim et al. [35] found that PEG-melittin-dKLA 8–26 significantly outperformed melittin-dKLA in controlling tumor growth, improving survival rates, and reducing lung metastasis in a TNBC model. This suggests that PEGylation enhances the stability and therapeutic potential of melittin-dKLA. Future research could focus on detailed pharmacokinetic and pharmacodynamic studies to optimize dosing regimens and administration routes for PEG-melittin-dKLA 8–26. Long-term studies could assess the potential for developing resistance or altering tumor microenvironment dynamics. Additionally, exploring the synergistic effects of PEG-melittin-dKLA 8–26 in combination with other novel therapeutic agents, such as chimeric antigen receptor (CAR)-T cell

therapies or immune checkpoint inhibitors, could offer new insights into its potential for combination treatments. Understanding how PEGylation affects the drug’s interaction with various biological targets could lead to improved formulations and therapeutic strategies.

Jia et al. [34] demonstrated that pacMEL showed approximately a 10-fold increase in area under the curve (AUC) and significantly reduced hepatic damage and hemolytic activity compared to free melittin. PacMEL also enhanced tumor suppression in a model with various tumor sites. Future research could focus on developing pacMEL formulations tailored for different types of cancers or specific patient populations, potentially broadening its application. Investigating the impact of pacMEL on cancer stem cells could provide valuable insights into overcoming drug resistance and expanding its therapeutic potential. Additionally, studying the effects of pacMEL on normal tissues and organs in animal models could help in minimizing off-target effects and improving safety profiles, thus facilitating its clinical translation.

Sahsuvar et al. [36] developed targeted and redox-responsive melittin conjugates that demonstrated effective anticancer activity, reduced hemolytic activity, and provided additional antioxidant and

Table 2
Summary of Preclinical Studies on Apamin-Based Therapeutics for Cancer Treatment.

References	Objective	Model	Intervention	Endpoints	Results	Conclusion	Possible Clinical Implications	Study's Limitations
Awan et al. [18]	Improve therapeutic effectiveness of 2ME using APA and INVA	A549 cells (in vitro)	INVA-APA or 2ME-INVA-APA at 0.39–100 μM for 48 hours	IC ₅₀ , cell death, necrosis, Bax/Bcl-2 expression, mitochondrial membrane potential, cell cycle analysis, apoptosis markers	2ME-INVA-APA showed an IC ₅₀ of 1.15 $\mu\text{g/mL}$, higher cell death and necrosis, G2-M phase arrest, increased apoptotic markers	2ME-INVA-APA enhances 2ME's effectiveness by inducing apoptosis and improving therapeutic potential	Could lead to more effective treatments for lung cancer with reduced doses and side effects; enhances drug targeting and delivery	The transition from in vitro to clinical trials may face challenges; long-term safety and efficacy in humans need assessment
Alhakamy et al. [24]	Develop and optimize APA and ALS-NC for pancreatic cancer therapy	PANC-1 cells (in vitro)	APA-ALS-NC at different concentrations based on IC ₅₀ for 24 hours	IC ₅₀ , necrosis phase, G2-M phase, mitochondrial membrane potential, apoptosis markers (IL-1 β , TNF- α , CAS-3, ROS, IL-6, NF- κB), gene expressions (Bax, Bcl-2, p53)	APA-ALS-NC had an IC ₅₀ of 1.01 $\mu\text{g/mL}$, higher necrosis, G2-M phase arrest, and enhanced apoptosis compared to ALS and APA	APA-ALS-NC demonstrates superior cytotoxicity and apoptotic effect, making it a promising candidate for pancreatic cancer therapy	Potential to improve outcomes in pancreatic cancer treatment with a more targeted and less toxic approach; could enhance patient survival	In vitro results need validation in preclinical and clinical studies; potential off-target effects and long-term safety are not yet known
Badr-Eldin et al. [25]	Investigate the potential of APA-functionalized EML to enhance EGA's cytotoxic activity against breast cancer	MCF-7 cells (in vitro)	EGA-EML-APA at 0.4–100 $\mu\text{g/mL}$ concentrations for 24–48 hours	IC ₅₀ , cell cycle arrest, apoptosis markers (p53, Bax, CAS-3, Bcl-2), NF- κB activity, TNF- α expression	EGA-EML-APA showed an IC ₅₀ of 5.472 $\mu\text{g/mL}$, G2-M and S phase arrest, increased apoptotic markers, abolished NF- κB activity	EGA-EML-APA is a promising anti-breast cancer formulation with enhanced cytotoxicity and apoptosis induction	May offer improved efficacy for breast cancer treatment and better targeting of cancer cells, potentially reducing systemic side effects	In vivo efficacy and safety need to be thoroughly evaluated; optimization parameters may need further exploration in clinical settings

Abbreviations: 2ME, 2-Methoxyestradiol; A549, Alveolar Basal Epithelial Adenocarcinoma Cells; ALS, Alendronate Sodium; APA, Apamin; Bax, Bcl-2 Associated X; Bcl-2, B-Cell Lymphoma Protein 2; CAS, Caspase; EGA, Ellagic Acid; EML, Emulsomes; IL, Interleukin; INVA, Invasomes; MCF-7, Human Breast Cancer; NC, Nanocomplex; NF- κB , Nuclear Factor Kappa B; p53, Tumor Protein p53; PANC-1, Pancreatic Cancer Cell Line; ROS, Reactive Oxygen Species; TNF- α , Tumor Necrosis Factor Alpha.

antibacterial benefits. These conjugates were tested in vitro on human breast cancer (MCF-7), HPV (–) human cervical squamous carcinoma (C33A), and HPV (+) human cervical adenocarcinoma (HeLa) cell lines. Future research could investigate the mechanisms behind the redox-responsive nature of these conjugates and their potential to be used in combination with other redox-active therapies. Exploring the application of these conjugates across different cancer types with varying oxidative stress profiles could reveal their broader applicability. Additionally, assessing the potential of these conjugates in targeting cancer-associated infections or enhancing immune responses could open new therapeutic avenues, potentially leading to multifunctional cancer treatments.

Awan et al. [18] improved the effectiveness of 2ME by using apamin and INVA, resulting in an IC₅₀ of 1.15 $\mu\text{g/mL}$, increased cell death, necrosis, G2-M phase arrest, and enhanced apoptotic markers in lung cancer cells. Future studies could focus on developing 2ME-INVA-apamin formulations for other types of cancer, examining their effects on different cancer cell lines and in vivo models. Investigating the potential for combining 2ME-INVA-apamin with novel targeted therapies or immunotherapies could provide insights into synergistic effects and optimized treatment regimens. Additionally, exploring the molecular mechanisms underlying the improved therapeutic efficacy and reduced side effects could guide the development of similar formulations for other drugs, potentially enhancing the overall effectiveness of cancer treatments.

Alhakamy et al. [24] developed apamin and alendronate sodium nanocomplexes that showed superior cytotoxicity, with an IC₅₀ of 1.01 $\mu\text{g/mL}$, higher necrosis, and enhanced apoptosis compared to individual components. Future research could explore the optimization of these nanocomplexes for improved targeting and reduced off-target

effects. Investigating the use of these nanocomplexes in combination with other therapeutic modalities, such as radiation therapy or targeted inhibitors, could reveal potential synergistic effects. Additionally, examining the pharmacokinetics and biodistribution of these nanocomplexes in various animal models could provide insights into their potential for clinical translation and safety profiles, potentially improving outcomes in pancreatic cancer treatment.

Badr-Eldin et al. [25] investigated apamin-functionalized emulsomes to enhance ellagic acid's cytotoxicity against breast cancer, achieving an IC₅₀ of 5.472 $\mu\text{g/mL}$ and increased apoptosis with cell cycle arrest. Future research could focus on developing similar apamin-functionalized formulations for other chemotherapeutic agents or natural compounds. Studying the impact of these formulations on different breast cancer subtypes, particularly those resistant or recurrent, could provide valuable insights. Additionally, exploring the use of apamin-functionalized emulsomes in combination with other therapeutic strategies, such as hormone therapy or targeted therapies, could offer new approaches to overcoming treatment resistance and improving patient outcomes, potentially leading to more effective and personalized cancer treatments.

In addition to the specific research directions for each study, there are several broader future research avenues that warrant exploration. Integrating genetic studies into the research on melittin- and apamin-based therapies could offer significant insights into how these treatments interact with specific genetic mutations or expressions associated with various cancers. Identifying genetic biomarkers that predict responses to these therapies could enable the development of more personalized treatment strategies, improving efficacy and minimizing adverse effects. Additionally, understanding the genetic mechanisms underlying resistance to these therapies could provide crucial

information for overcoming treatment challenges.

Advancing immunological therapies represents another promising direction. Future research could explore how melittin- and apamin-based therapies might enhance or complement existing immunotherapies, such as checkpoint inhibitors, CAR-T cell therapies, or cancer vaccines. Assessing how these peptides could modulate immune cell activity and the tumor microenvironment could lead to novel combination strategies that maximize the potential of both direct peptide effects and the immune system's ability to combat cancer. Melittin has significantly emerged as a promising candidate for enhancing cancer immunotherapy. This bee venom-derived peptide is promising due to its ability to not only induce immunogenic cancer cell death but also promote the maturation of antigen-presenting cells and induce the activation of T cells while promoting the enhancement of the infiltration and cytotoxicity of effector lymphocytes and modulating macrophage phenotypes for relieving immunosuppression [50]. Over the past few years, immunotherapy against cancer has demonstrated encouraging outcomes in enhancing treatment efficacy against different cancer lineages. Immunotherapy also offers a viable strategy for enhancing the life quality of cancer patients [51,52]. Combining immunotherapy with apamin can also be a great avenue to mitigate adverse events or limit costs. Apamin could sensitize the cancer cells to immunotherapy, especially those presenting a different conformation of ion channel profile. Current research has addressed the roles of ion channels in chemo-resistance [53]. By sensitizing cancerous cells, immunotherapy efficacy might increase even more, and fewer sessions might be necessary to complete treatment. In this scenario, future research endeavors must prioritize introducing apamin combined with immunotherapies to target cancerous lines.

Personalizing treatment approaches based on individual patient characteristics is crucial for improving therapeutic outcomes. Research could focus on tailoring melittin- and apamin-based therapies to specific tumor profiles, including genetic, molecular, and phenotypic characteristics. This could involve developing assays to match these peptide therapies to patients who are most likely to benefit, potentially enhancing treatment efficacy and reducing the likelihood of adverse effects. In personalized treatment strategies against cancer, it is known that specific cancer types present a more specific ion channel profile than others. In this scenario, targeting cancerous cells with a prominent calcium-activated potassium channel expression with apamin is of utmost potential. Calcium-activated potassium channels have been assigned to specific biological functions within cancerous cells. These channels partially enhance cancerous cell proliferation and migration [54]. By using immunohistochemistry analyses of even patch clamps or high-throughput analysis, biomedical researchers can determine the content of calcium-activated potassium channels of a cancer cell line and prioritize treatment based on personalization. This can effectively and significantly mitigate cancer cell growth and reduce the migration and occurrence of metastasis [55]. Apamin was also used in a disulfide-linked α -helix-containing peptide to mimic the extracellular, specifically, the structured N-terminal part of the protein p32, and serve as an imprinting template for generating a polymeric nanoparticle that recognizes and binds to p32-positive tumor cells. This has the potential to mediate targeted photodynamic therapy in vivo successfully and actively target tumors through conformational epitope imprinting [56]. Melittin has also been evaluated using specific personalized treatment strategies. Adenovirus expressing apoptin and melittin genes were used to kill hepatocellular carcinoma cells and inhibit ectopic tumor growth [57].

Exploring the synergistic effects of melittin- and apamin-based therapies with traditional chemotherapy and radiotherapy agents could also offer new avenues for research. Investigating how these peptides interact with standard treatments to enhance their effectiveness or reduce their side effects could lead to more comprehensive and effective cancer treatment regimens. Research could focus on optimizing combination therapies to improve overall treatment outcomes,

potentially overcoming limitations associated with current therapies and offering new hope for patients with challenging or resistant cancer types. Melittin was found to have radiosensitizing effects on esophageal squamous cell carcinoma and prominent apoptosis induction in vitro and in vivo. Melittin successfully sensitized esophageal squamous cell carcinoma cells to radiation therapy with an utmost 1.15–1.42 sensitization enhancement ratio. Besides, radiosensitization was accompanied by enhanced apoptosis [58]. Melittin also radiosensitized hypoxic head and neck squamous cell carcinoma by suppressing hypoxia-inducible factor 1- α (HIF-1 α). Melittin treatment also resulted in cancerous cell growth inhibition, cancerous cell induction of apoptosis, and reduced vascular endothelial growth factor (VEGF) expression [59]. Recently, progress has been made in radiotherapy against multiple cancer lineages. Radiotherapy treatment modality is up to rive cancer cells of their multiplication potential, decreasing cell division. Although progress has shaped radiotherapy to decreased side effects and enhanced patient survival, it is known that this is one of the primary cancer treatment options leading to side effects, which may often diminish patients' quality of life [60]. In order to prevent the occurrence of adverse effects associated with radiotherapy, the possible synergism between radiation and apamin derived from bee venom must be addressed in future research. Cancer cells may exhibit different profiles of ion channels [61]. Apamin treatment against cancer cells that present different conformations of ion channel profiles may offer a nuanced and improved radiotherapy approach. Apamin may limit cancer cell survival by modulation of ion channels, especially in chemo-resistant tumors. This may not only increase cancerous cell susceptibility to radiation but also decrease the necessary number of radiotherapy sections to treat cancer.

Currently, there are no studies evaluating apamin and melittin's synergistic effects. The current published studies did not evaluate the potential synergistic effects of apamin and melittin separately from other compounds naturally encountered in bee venom. This can also be another promising area of research. However, caution is warranted due to the possible unintended effects of one on another's effectiveness. While apamin selectively targets the calcium-dependent potassium channels to inhibit it, melittin disrupts the cell membrane via melittin peptides' oligomerization. Calcium-dependent potassium channels are localized at the internal surface of the cell membrane [62]. Melittin disrupts cell membranes, leading to the leakage of cellular components [63]. Therefore, synergizing apamin and melittin may not be a good strategy for combating cancer cells due to possible wastage of resources.

Whole bee venom is indeed used nowadays to treat specific types of cancer, such as gastrointestinal, pancreatic, gynecological, leukemia, hepatic, lung, and many others. However, it is crucial to note that only 3 % of bee venom comprises apamin. On the other hand, 57 % correspond to melittin. Other components comprise phospholipase A2 (11 %), hyaluronidase (2 %), MCD-peptide (3 %), and adolapin (1 %). Unspecified components comprise 23 % [17]. Therefore, conclusions of a potential synergism between apamin and melittin are challenging to reach based on the current literature.

Despite the promising potential of melittin-based therapies, challenges related to melittin toxicity remain a significant concern. Melittin, while effective against cancer cells, can also exhibit cytotoxic effects on healthy tissues, limiting its clinical applicability. Future research should focus on developing strategies to mitigate these toxic effects. One approach could involve engineering melittin derivatives with enhanced selectivity for cancer cells, potentially reducing off-target toxicity. Another avenue is the use of advanced delivery systems, such as targeted nanoparticles or liposomes, to confine melittin's activity to tumor sites and minimize systemic exposure. Additionally, exploring combination therapies that incorporate melittin with other agents designed to protect normal cells or modulate the tumor microenvironment could offer a balanced approach to leveraging its therapeutic benefits while managing toxicity. Addressing these challenges is crucial for translating melittin-based therapies from preclinical success to effective clinical

treatments.

These broader research directions could significantly advance the field of cancer treatment, leading to more effective, personalized, and comprehensive therapeutic strategies.

5. Toxicity, safety, and other considerations for apamin and melittin-based therapies

Lallement et al. [64] investigated the toxicity of apamin using male Wistar rats with both natural and synthetic forms of the peptide. They found that apamin caused notable signs of toxicity, including ataxia and tremor, with the severity increasing with the dose. The estimated lethal dose (LD₅₀) for apamin administered intraperitoneally was 2.05 mg/kg. Animals treated with apamin displayed significant pulmonary issues, such as hyperinflation and petechial hemorrhages, as well as congestion in the liver, spleen, kidneys, and adrenal glands. Histological examinations revealed severe intra-alveolar hemorrhage and congestion in the lungs, along with focal alveolar edema and fibrin deposition. The study also noted that animals receiving apamin via intracerebroventricular injection experienced quicker onset of symptoms than those receiving intraperitoneal injections. Additionally, Jullié et al. [65] evaluated the toxicity of various apamin analogs by intracerebroventricular injection into mice and calculated the LD₅₀ values for each. The LD₅₀ of native apamin was determined to be 12 ng. Apamin(1–15) and apamin(1–16) exhibited significantly higher LD₅₀ values of 2800 ng and 3400 ng, respectively, indicating reduced toxicity compared to the native peptide. The analogue [Ala-10]apamin had an LD₅₀ of 18 ng, showing slightly increased toxicity relative to apamin(1–15) and apamin(1–16). Apamin(1–17) had an LD₅₀ of 75 ng, demonstrating a notable decrease in toxicity compared to native apamin but still higher than [Ala-10]apamin. The [cHxO₂]apamin analogue, which is chemically modified at the arginine residues, exhibited the highest LD₅₀ value, indicating the lowest toxicity among the tested analogues.

While melittin is cytotoxic to a wide range of tumor cells, it also exhibits toxicity toward normal cells. Its utility is limited by its non-selective nature; melittin does not only target cancerous cells but also exhibits toxicity toward normal, healthy cells. This lack of specificity can result in adverse effects and damage to normal tissues, which poses a challenge in using melittin as a therapeutic agent without causing collateral harm. Melittin has been demonstrated to be toxic to various hematopoietic cells, including lymphocytes, thymocytes, and erythrocytes, as well as to intestinal cells. Melittin also affects numerous cellular metabolic processes and disrupts the plasma membrane, leading to alterations in the enzymatic system. Additionally, its lytic activity is linked to its ability to integrate into the phospholipid bilayers of cell membranes [66,67]. In Askari et al.'s study [68], melittin's toxicity was evaluated in male BALB/c mice. The acute LD₅₀ was 4.95 mg/kg, with a maximum sub-lethal dose of 2.4 mg/kg. In sub-acute tests, mice received melittin at 2.4 mg/kg every 12 or 8 hours for five days. While mice survived, they showed temporary pain symptoms post-injection. Biochemical tests showed no significant changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, or creatinine. However, white blood cell (WBC) counts were significantly lower in melittin-treated mice. Liver and kidney weights and histopathology revealed no significant damage. Gajski et al. [67] demonstrated that melittin is cytotoxic to human peripheral blood lymphocytes (HPBLs) through several molecular mechanisms. At doses of 1 and 5 µg/mL, melittin induces significant oxidative stress, as evidenced by increased reactive oxygen species (ROS) formation and reduced glutathione levels. Elevated lipid peroxidation was observed, contributing to deoxyribonucleic acid (DNA) damage, including oxidative damage, micronuclei formation, and nuclear buds. The study also showed that melittin affects gene expression, altering the levels of genes involved in DNA damage response [tumor protein p53 (p53), cyclin dependent kinase inhibitor 1A (CDKN1A), growth arrest and DNA-damage-inducible alpha (GADD45α), transformed 3T3 cell double minute 2 (MDM2)],

oxidative stress [catalase (CAT), superoxide dismutase type 1 (SOD1), glutathione peroxidase 1 (GPX1), glutathione-disulfide reductase (GSR), and glutamate-cysteine ligase catalytic subunit (GCLC)], and apoptosis [Bcl-2, Bax, CAS-3, and CAS-7]. These findings indicate that oxidative stress is a key factor in melittin genotoxic effects and highlight the need to consider melittin toxicity to normal cells in therapeutic contexts.

In our study, mainly apamin conjugates exerted cancer cell apoptosis via the mitochondrial and DR pathways. Awan et al. [18] and Alhakamy et al. [24] demonstrated apamin conjugate effects on Bax/Bcl-2 expression and mitochondrial membrane potential damage related to mitochondrial apoptotic pathways. Alhakamy et al. [24] and Badr-Eldin et al. [25] demonstrated apamin conjugates potential to induce mitochondrial apoptosis via CAS-3 and p53 regulation. Through DR pathways, these authors demonstrated apamin's ability to induce apoptosis, possibly via the TNF-α receptor pathway. Additionally, apamin can modulate NF-κB and, therefore, possibly induce cancer cell apoptosis via other DR, as the above-mentioned authors indicated.

Since apamin and melittin are toxins and derived from bee venom, it can be supposed that these molecules may exert toxic interaction with other cells instead of only targeting cancerous cells [69]. Although specific studies regarding predilection of action have not yet been conducted, there are suppositions based on apamin and melittin mechanisms of action. Firstly, it is noteworthy that tumor cells may present increased sensitivity to apamin because these cells often possess altered ion channel profiles [70]. It is also possible that apamin may possess a nuanced interaction with cancerous cells due to their increased expression of such channels as calcium-dependent potassium channels [71], which are the main targets of apamin [72]. Regarding melittin, it is known that its mechanism of action may be relatively less specific than that of apamin. However, it is also known that cancerous cells may possess altered membrane lipid compositions compared to normal and healthy cells [73]. Due to altered lipid compositions, cancer cells may also present altered membrane function [74,75], leading to altered membrane permeability [76]. This altered membrane function and permeability can predispose cancerous cells to be more susceptible to melittin's effects. It is also possible that cancerous cells might be more susceptible to melittin's effects on pore formation due to their rapid growth, leading to an increased membrane turnover [77]. Using melittin as adjuvant cancer treatment is particularly promising because physical damage induced by pore-forming peptides naturally encountered may be a great strategy to overcome most of the resistance mechanisms of tumor cells, including increased externalized phosphatidylserine [78] and membrane resealing [79]. Melittin's ability to influence pore formation via direct interaction with membrane lipids may overcome these resistance mechanisms more effectively than other naturally occurring pore-forming peptides interacting with specific membrane proteins.

Apamin and melittin are extracted after venom collection, which happens in a controlled manner without causing harm to the bees. The collection of the venom can usually be carried out through gland extraction or electrostimulation [80,81]. After collection, techniques such as high-performance liquid chromatography (HPLC) coupled with mass spectrometry are used for the separation and purification of both apamin and melittin with a high degree of purity [82]. Melittin can also be separated and purified by ion exchange chromatography [83] or high-resolution reversed-phase liquid chromatography [21,84].

Melittin can stay stable as a solution at four °C in the refrigerator for at least eight weeks [85]. On the contrary, apamin is less stable under positive temperatures and must be usually stored at temperatures ranging from –70°C [86]. The peptide structure of apamin and melittin makes them susceptible to protease degradation. Adding a non-cationic amino acid to the peptide chain could prevent this process, increasing the half-life time [87]. Apamin and melittin are commercialized under high-purity conditions. Rapid search under reputable pharmaceuticals can demonstrate HPLC apamin [88] and melittin [89] being sold with a 95 % or higher purity level.

Apamin appears to be excreted by the kidney. Regarding

metabolization, apamin was degraded in vitro by homogenates derived from the liver, the kidneys, and the brain. In a study by Cheng-Raude et al., the kidneys possessed the highest content in apamin-sized labeled peptides and the highest specific radioactivity after in vitro and in vivo studies [90]. Data regarding melittin's metabolization and excretion from the body has not yet been published.

6. Conclusions

In conclusion, this systematic review provides a thorough analysis of the therapeutic potential and progress of bee venom-derived apamin and melittin conjugates in cancer treatment (Fig. 4). By evaluating a range of preclinical studies, the review highlights significant advancements in peptide-based therapies, including their enhanced efficacy and minimized side effects. The review offers valuable insights into the mechanisms of action and outlines critical future research directions needed to translate these findings into clinical practice.

In the included studies, many were the reported mechanisms by which apamin and melittin conjugates could inhibit cancer cell growth and metastasis. Apamin conjugates suppressed cancer cells due to a significant increase in Bax expression, reduced Bcl-2 expression, and mitochondrial membrane potential loss. Apamin conjugates also

induced G2-M phase arrest in cancer cells, upregulated CAS-9 and p53, and downregulated NF- κ B [18]. Apamin conjugates also enhanced apoptosis and decreased cancer cell growth via modulation of interleukin (IL)-1 β , TNF- α , CAS-3, ROS, IL-6, NF- κ B levels, Bax, Bcl-2, and p53 levels [24]. These effects were confirmed by other studies [25]. On the other hand, melittin conjugates promoted cancer cell growth and metastasis inhibition due to principally reduced M2-like TAMs [35] and cancer cell growth inhibition via modulation of NK cell infiltration [49]. Unfortunately, two studies on melittin conjugates that were included did not report specific mechanisms of cancer cell growth and metastasis suppression. Additionally, just one study on melittin conjugates demonstrated the effects of the bee venom peptides against cancer cell metastasis. Observing the included studies, it is possible to conclude that the mechanisms by which apamin and melittin conjugates suppress cancer cell growth are slightly different. While apamin conjugates appear to be effective mainly by modulating molecular pathways that are intrinsically related to cancerous cells, melittin conjugates rely on suppressing M2-like TAMs and modulating NK cell infiltration.

A key strength of the review is its detailed examination of innovative approaches to peptide conjugates, such as their integration with advanced delivery systems and targeting strategies. These developments are crucial for improving the effectiveness of cancer treatments and

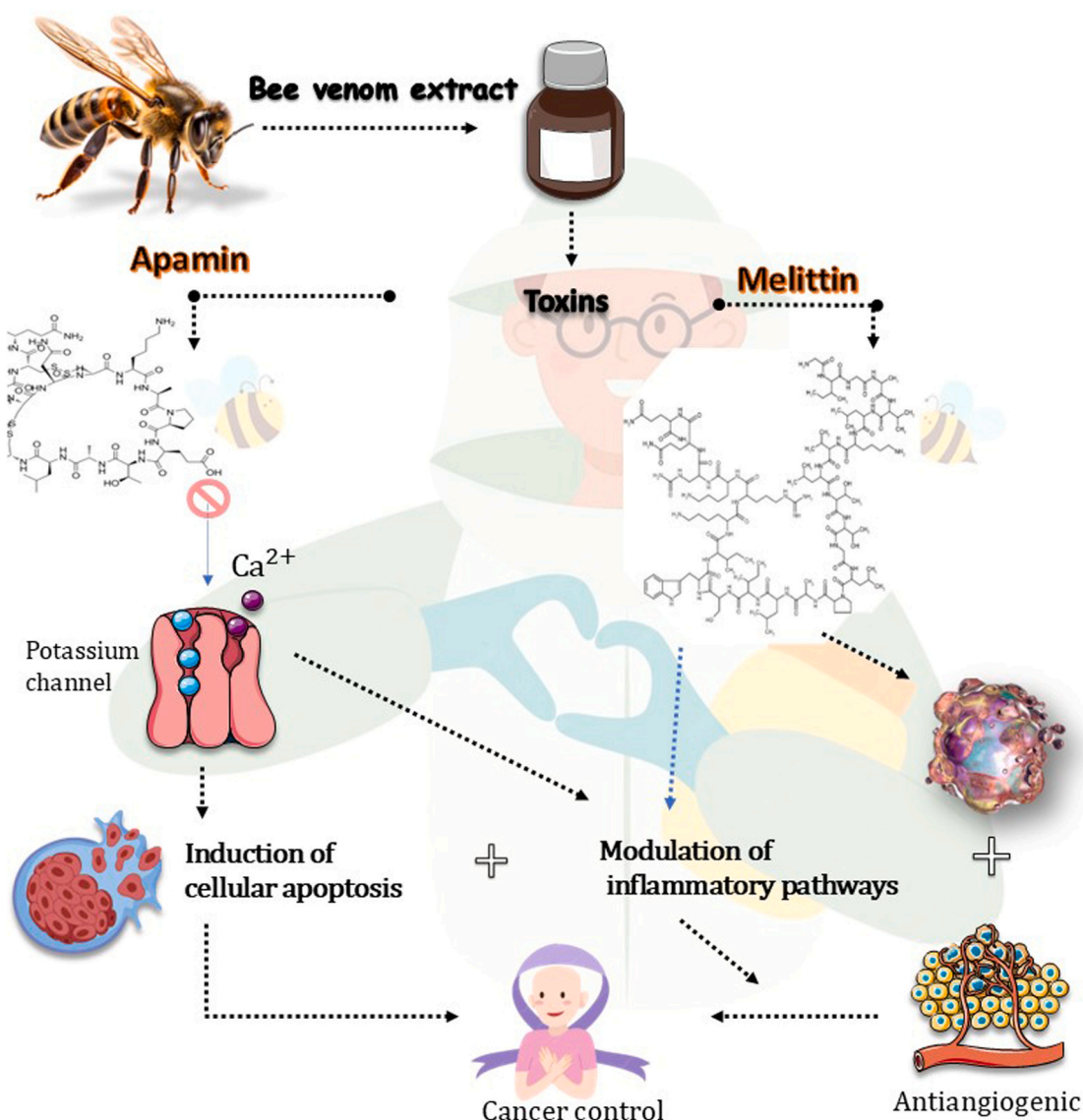


Fig. 4. Therapeutic Potential of Bee Venom-derived Apamin and Melittin Conjugates Against Cancer.

aligning them with personalized medicine principles. The potential for these conjugates to offer more tailored and effective treatments could significantly enhance patient outcomes.

However, the review also acknowledges limitations, including variability in study methodologies and cancer models, which may complicate the comparison and generalization of results. Furthermore, while preclinical findings are promising, substantial challenges remain in translating these therapies into clinical practice. Standardized and rigorous clinical trials are essential to confirm the safety and efficacy of these therapies in human populations. Additionally, knowing that data on melittin's metabolism and excretion from the body has not been extensively published, it is crucial that future research must endeavor to comprehend its metabolism and elimination to prevent off-targets and potentially severe side effects in patients with liver or kidney diseases.

Introducing bee venom-derived therapies into cancer treatment offers promising therapeutic benefits and aligns with green healthcare principles [91]. Green healthcare emphasizes environmentally friendly practices, which the use of natural substances like bee venom can exemplify. Incorporating such biocompatible and sustainable sources into cancer treatment respects environmental considerations and can reduce the reliance on synthetic chemicals. For example, utilizing bee venom-derived peptides can minimize the environmental impact associated with producing and disposing of conventional pharmaceutical agents. As highlighted in the Institute of Medicine (IOM) Roundtable workshop, green healthcare is compelling for its potential to directly and indirectly protect and promote health. By incorporating bee venom-derived apamin and melittin conjugates into cancer treatment, we advance personalized cancer therapies and adhere to environmentally responsible practices that can contribute to broader health and sustainability goals. As research progresses, it will be essential to continue exploring how these therapies can be optimized and expanded, ensuring that they offer significant clinical benefits and contribute to a more sustainable and responsible approach to cancer care.

Ethical approval

Not applicable.

Statement of Human and Animal Rights

This article does not contain any studies with human and animal subjects performed by any of the authors.

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Declaration of Competing Interest

The authors confirm that the manuscript is an original work and has not been published previously in any form or any other journal.

Furthermore, the authors affirm that this manuscript is not currently under review or consideration for publication by any other journal. All authors involved in this research have thoroughly reviewed and approved the manuscript in its current form and consent to its submission to the journal *Pharmacological Research*.

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On behalf of all the co-authors agreement, the Corresponding Author signs.

Thank you for taking the time to consider our manuscript for publication in *Pharmacological Research*. The authors appreciate the opportunity to present their research to your esteemed journal. The authors are confident that the findings and insights reported in their manuscript will make a meaningful and significant contribution to the field of pharmacology.

Data Availability

Data will be made available on request.

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