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Review Article

Anticancer Properties of Bioactive Compounds Present in the Sting of Wild Honey Bees (*Apis dorsata*): A Conceptual Review

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ABSTRACT

Cancer is one of the leading causes of illness and death worldwide. This has prompted ongoing research into new therapeutic drugs and treatment techniques. In this context, natural products have received much interest because of their ability to battle cancer with fewer side effects than conventional chemotherapy. Among these natural sources, honey bee products, notably venom, have demonstrated promising anticancer activity. The sting of the wild honey bee, Apis dorsata, a species known for its potent venom, contains a complex mixture of bioactive compounds that may offer unique anticancer benefits. Bee venom, also known as apitoxin, comprises various enzymes, peptides and amines, including melittin, phospholipase A2 and apamin. These compounds have been extensively studied for their pharmacological activities, including anti-inflammatory, antimicrobial and anticancer effects. Recent studies suggest that these components can induce apoptosis (programmed cell death), inhibit tumor growth and enhance the efficacy of other anticancer drugs. However, much of the existing research has focused on bee venom from species such as Apis mellifera, with limited studies specifically addressing the venom of A. dorsata. Given the distinct ecology and behavior of A. dorsata, which often result in different venom compositions, it is necessary to explore its specific anticancer potential. This concept review seeks to provide a comprehensive and existing knowledge in the field of anticancer properties of bee venom, with a focus on the chemicals present in the sting of A. dorsata. By analyzing the unique components of this venom and their potential mechanisms of action, this review highlights the potential of A. dorsata venom as a source of novel anticancer agents.

***Keywords**: Apis dorsata, Bee venom, Anticancer properties, Melittin, Phospholipase A2.*

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INTRODUCTION

Cancer remains a significant global health challenge, driving the ongoing search for novel therapeutic agents with high efficacy and minimal side effects. Natural products, particularly those derived from insects, have gained considerable attention in recent years for their potential in cancer therapy. Among these, bee venom has emerged as a promising source of anticancer agents due to its complex mixture of bioactive compounds. The venom of the wild honey bee, *A. dorsata* (Figure 1), is particularly noteworthy given its potent pharmacological properties. Bee venom (BV), or apitoxin, is composed of a variety of enzymes, peptides and small molecules, including melittin, phospholipase A2 and apamin, which have demonstrated significant biological activities. Melittin, the main component of bee venom, is known for its ability to induce apoptosis (programmed cell death) in cancer cells, disrupt cell membranes and inhibit tumor growth. Similarly, phospholipase A2 has been shown to exhibit cytotoxic effects on cancer cells, enhancing the anticancer potential of the venom (Son et al., 2007; Oršolić, 2012; & Gajski et al., 2024). Research into BV has largely focused on *Apis mellifera*, with limited studies on the venom of *A. dorsata*. However, given the distinct ecological niche and aggressive behavior of *A. dorsata*, which may result in unique venom composition, it is essential to explore its potential as a source of novel anticancer agents. This review aims to synthesize current knowledge on the anticancer properties of bee venom, with a particular emphasis on the bioactive compounds present in the sting of *A. dorsata* and their potential mechanisms of action (Lee et al., 2016; Zhang et al., 2018; & El Mehdi et al., 2022). By delving into the unique aspects of *A. dorsata* venom, this review seeks to highlight its potential role in the development of innovative cancer therapies.

Figure 1: ***Apis dorsata***

Composition of Bee venom: Bee venom, also known as apitoxin, is a complex mixture of proteins, peptides and low molecular weight components (Figure 3). It plays a crucial role in the defence mechanism of bees and has been studied for its potential therapeutic properties. Below is a detailed breakdown of the main components of bee venom (Ali, 2012; Habermann, 1972; Dotimas & Hider, 1987; & Pandey et al., 2023):

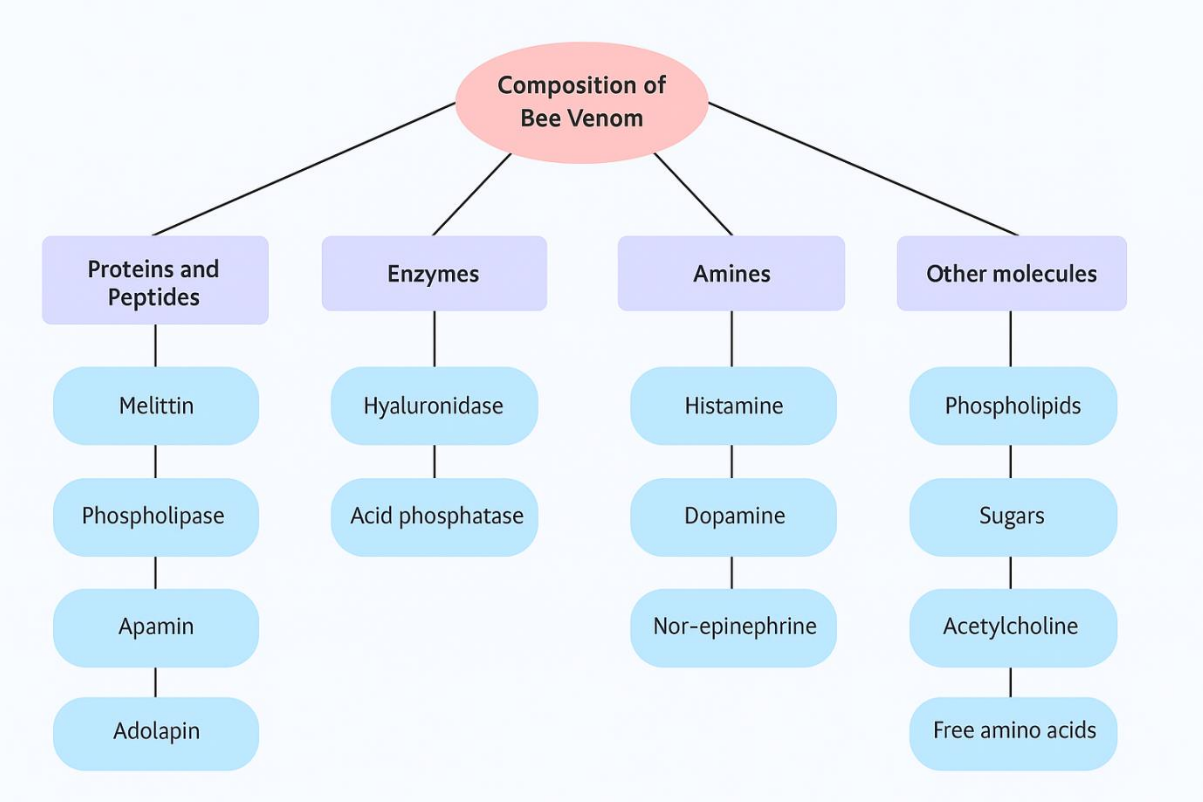

Figure 2: Composition of Bee Venom

<u>1.</u> <u>Proteins and Peptides:</u>

(A): Melittin: This is the most abundant peptide in bee venom, constituting 40-60% of the dry weight. Melittin is a potent anti-inflammatory and antimicrobial agent, but it is also responsible for the pain and swelling associated with bee stings. It can disrupt cell membranes, leading to cell lysis. This small, amphipathic peptide consists of 26 amino acids and is well-known for its powerful biological activities, which are both beneficial and harmful depending on the context.

A-1: Structure and Mechanism of Action: Melittin has a unique structure, with a hydrophobic (water-repelling) tail and a hydrophilic (water-attracting) head. This structure allows it to insert into lipid bilayers of cell membranes, leading to their disruption. Upon insertion, melittin aggregates to form pores, causing cell lysis or apoptosis (programmed cell death). This pore-forming ability is responsible for the pain and damage associated with bee stings, but it also makes melittin a candidate for therapeutic applications, such as targeting cancer cells (Habermann, 1972) (Figure 3).

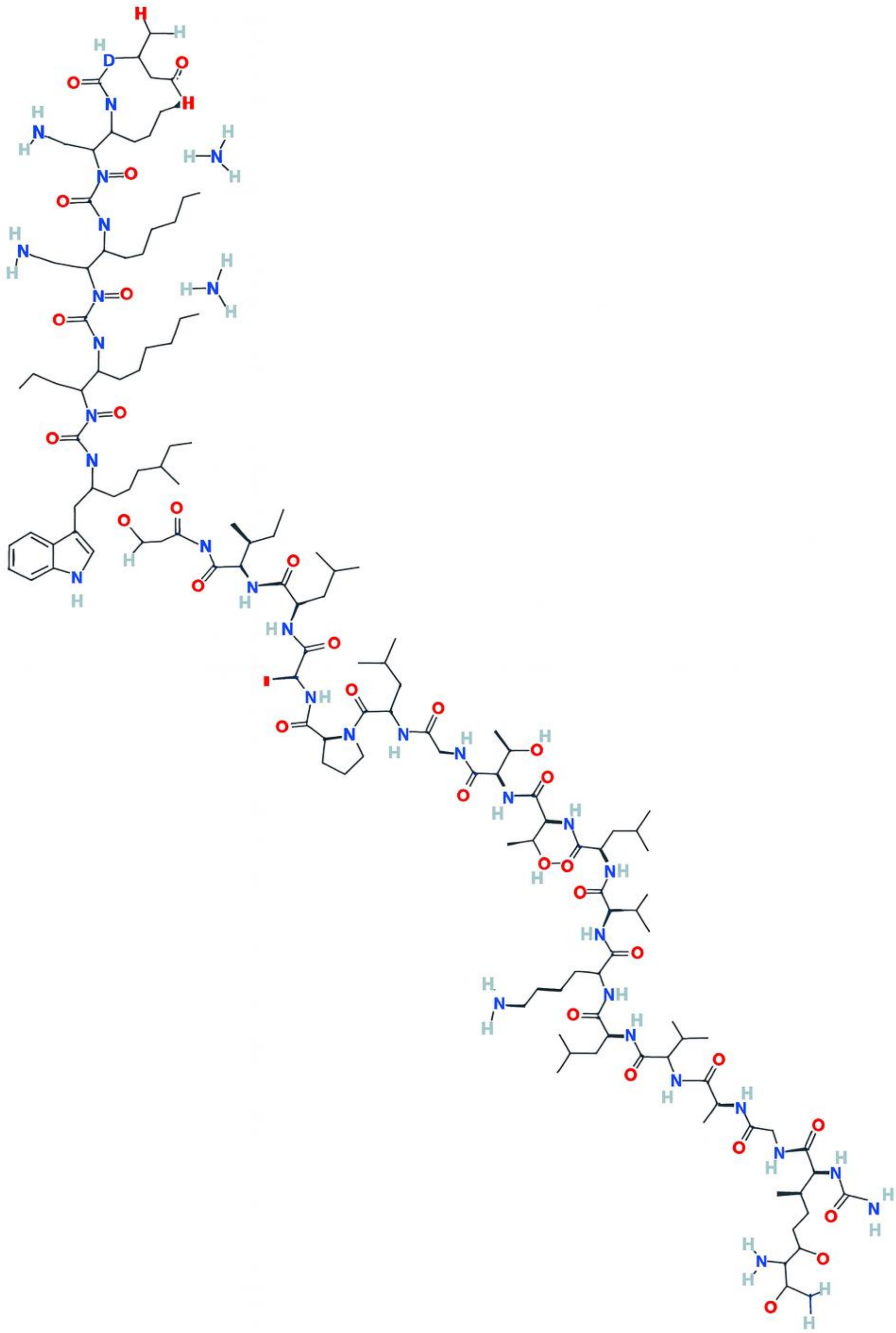

Figure 3: Structure of Melittin ($C_{131}H_{229}N_{39}O_{31}$), Source: PubChem (PubChem CID:16133648)

A-2: Anti-inflammatory and Antimicrobial Properties: Melittin has been shown to modulate the immune response by inhibiting the activity of enzymes like phospholipase A2, which are involved in the production of pro-inflammatory molecules. This anti-inflammatory effect is partly why bee venom therapy is used in conditions like rheumatoid arthritis. Additionally, melittin has demonstrated antimicrobial activity against a variety of bacteria, fungi and viruses, making it a potential candidate for treating infections.

A-3: Cytotoxicity and Cancer Research: Due to its ability to disrupt cell membranes, melittin exhibits cytotoxicity against a wide range of cell types, including cancer cells. Research has shown that melittin can induce apoptosis in cancer cells by disrupting mitochondrial membranes and activating apoptotic pathways. It has been studied in the context of various cancers, including breast cancer, melanoma and leukemia. Moreover, when conjugated with nanoparticles, melittin can be targeted more specifically to cancer cells, reducing its toxicity to normal cells.

A-4: Pain and Neurotoxic Effects: The pain from a bee sting is largely attributed to melittin's activation of pain receptors and the release of pro-inflammatory substances such as histamine from mast cells. Its interaction with nerve cells can also lead to neurotoxic effects, which have attracted significant research interest for understanding pain mechanisms and developing novel analgesics. Due to its broad spectrum of biological activities, this peptide has emerged as a promising candidate for therapeutic applications, particularly in cancer treatment, antimicrobial strategies and anti-inflammatory therapies. However, its non-selective cytotoxicity poses a considerable obstacle. To address this, scientists are investigating targeted delivery approaches—including liposomes, nanoparticles and peptide engineering—to improve specificity and reduce toxicity. As a bioactive compound, it represents a double-edged sword: its powerful effects offer exciting therapeutic potential but require careful handling to ensure safety and precision. Ongoing research is crucial to unlocking its potential while minimizing its harmful effects (Memariani et al., 2019; Raghuraman & Chattopadhyay, 2007).

B): Phospholipase A2 (PLA2): Making up about 10-12% of bee venom, PLA2 is an enzyme that breaks down phospholipids in cell membranes, leading to cell disruption and inflammation. It also enhances the effects of melittin. PLA2 is a significant enzyme found in the venom of the giant honeybee, *A. dorsata*. This enzyme plays a crucial role in the venom's toxicity and biological activity, contributing to both the immediate effects of a sting and its potential therapeutic applications (Kini, 1997).

(B.1): Structure and Function: PLA2 is a small, secreted enzyme that specifically hydrolyzes the sn-2 ester bond of phospholipids, producing free fatty acids and lysophospholipids. These products can have various downstream effects, including the activation of inflammatory pathways. The enzyme's structure typically includes a catalytic domain with a calcium-binding site that is essential for its enzymatic activity (Habermann, 1972) (Figure 4).

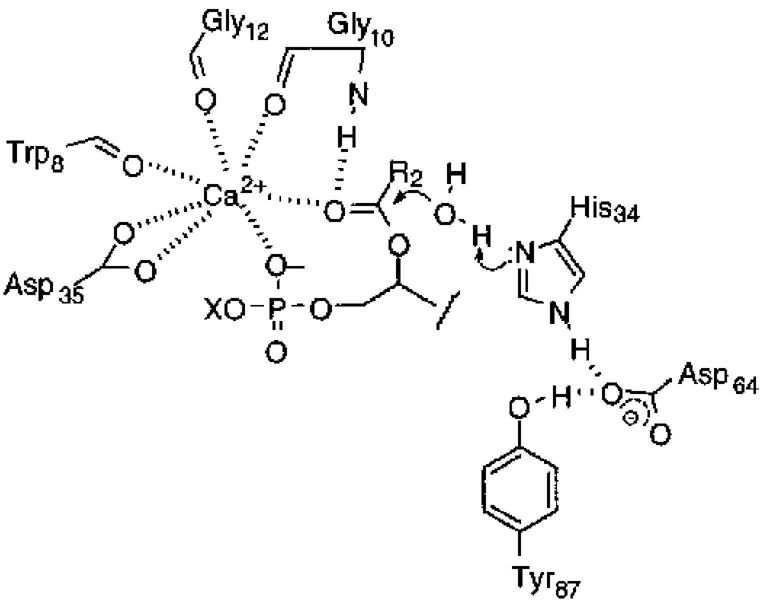

Figure 4: Structure of PLA2 (Annand et al., 1996).

(B.2): Biological Effects:

1. **Cell Membrane Disruption**: PLA2's primary function is to break down phospholipids in cell membranes, leading to membrane destabilization and cell lysis. This action amplifies the effects of other venom components, such as melittin, by making cell membranes more susceptible to damage.
2. **Pro-inflammatory Effects**: The hydrolysis of phospholipids by PLA2 leads to the release of arachidonic acid, a precursor for eicosanoids like prostaglandins and leukotrienes, which are potent mediators of inflammation. This is one of the reasons why bee stings cause pain, swelling and redness. Additionally, the enzyme can act directly on immune cells, promoting the release of histamine and other pro-inflammatory substances from mast cells and basophils.
3. **Neurotoxic and Hemolytic Activity**: PLA2 can exhibit neurotoxic effects by disrupting neuronal cell membranes, leading to altered nerve signaling. Its hemolytic activity, which involves the breakdown of red blood cells, further contributes to the toxic effects of bee venom.
4. **Synergistic Effects with Other Venom Components**: PLA2 works synergistically with other venom components, such as melittin, to enhance the overall toxicity of the venom. Melittin increases the permeability of cell membranes, making it easier for PLA2 to access and hydrolyze membrane phospholipids. This collaboration results in more effective cell damage and a stronger inflammatory response (Figure 5).

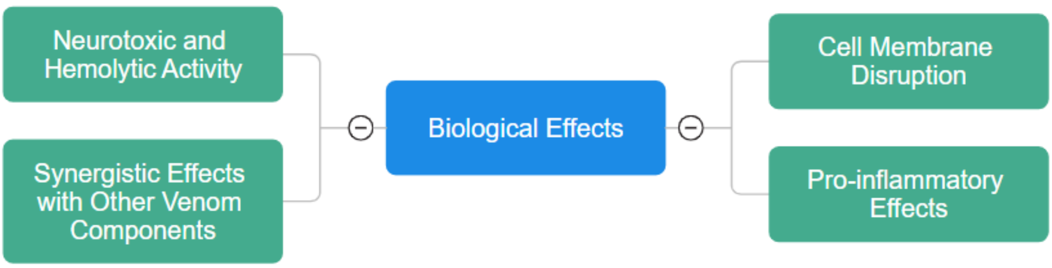

Figure 5: Biological effects of bee venom

PLA2 has attracted interest for its potential therapeutic applications, particularly in the treatment of inflammatory conditions and as an antimicrobial agent. Its ability to modulate immune responses makes it a candidate for treating diseases such as rheumatoid arthritis and other autoimmune disorders. However, its potent inflammatory effects also pose challenges, as uncontrolled PLA2 activity can exacerbate inflammation and tissue damage. Researchers are investigating ways to harness PLA2's beneficial properties while minimizing its harmful effects. This includes developing PLA2 inhibitors or designing delivery systems that target PLA2 activity to specific tissues or cells (Bon, 1997).

(B.3): Comparison with Other Species: The PLA2 enzyme in *Apis dorsata* shares similarities with PLA2 enzymes from other bee species, such as *Apis mellifera* (the European honeybee). However, there may be differences in enzyme activity, structure and the overall composition of the venom, reflecting the distinct ecological niches and evolutionary pressures faced by these species. Phospholipase A2 from *Apis dorsata* is a crucial component of the bee's venom, contributing significantly to its toxicity and inflammatory effects. While its primary role is to defend the bee and its colony, the enzyme also holds promise for therapeutic applications. However, further research is needed to fully understand its mechanisms and to develop safe and effective medical uses (Xu et al., 2010).

(C): Apamin: This peptide accounts for around 2-3% of bee venom. It is known for its neurotoxic effects and can block calcium-activated potassium channels, which has implications for neurological research. Apamin is a small, neurotoxic peptide found in bee venom, particularly from the species *Apis mellifera* (the European honeybee). It constitutes approximately 2-3% of the venom's dry weight and is one of the key components contributing to the venom's neurotoxic effects. Apamin is of significant interest in both medical research and toxicology due to its specific action on ion channels in the nervous system (Stocker, 2004).

(C.1): Structure and Mechanism of Action: Apamin is a peptide composed of 18 amino acids and its molecular structure includes two disulfide bridges that stabilize its configuration. The peptide is highly selective in its action, primarily targeting small-conductance calcium-activated potassium channels (SK channels) in the central nervous system (Figure 6).

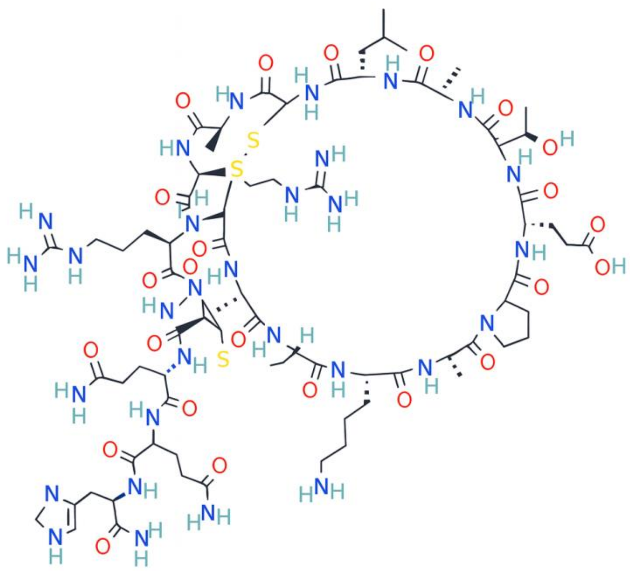

Figure 6: Structure of Apamin ($C_{79}H_{131}N_{31}O_{24}S_4$), Source: PubChem (16133797).

(C.2): Inhibition of SK Channels: Apamin blocks SK channels by binding to them and preventing potassium ions from exiting the neuron. SK channels are crucial for regulating the afterhyperpolarization phase following an action potential, which is a process that controls the frequency and pattern of neuronal firing. By inhibiting these channels, apamin increases the excitability of neurons, which can enhance synaptic transmission and potentially lead to neurotoxicity (Adelman et al., 2012).

(C.3): Biological Effects:

1. **Neurotoxic Effects**: Apamin's ability to block SK channels results in increased neuronal excitability, which can contribute to the neurotoxic effects of a bee sting. In higher concentrations, this increased excitability may lead to abnormal nerve signaling, muscle spasms and even convulsions.
2. **Potential Therapeutic Applications**:
   - Despite its neurotoxicity, apamin's precise action on SK channels has made it a valuable tool in neurological research. It is used experimentally to study the role in various physiological and pathological processes, including learning, memory and motor control.

- **Parkinson's Disease**: Some studies have suggested that modulating SK channel activity with apamin could have therapeutic potential in treating neurodegenerative conditions like Parkinson's disease by enhancing dopaminergic transmission.
- **Cognitive Function**: There is also interest in the role of SK channels in cognitive function, with research exploring whether apamin or similar molecules could enhance cognitive performance or treat cognitive deficits.

3. **Cardiovascular Effects**:

- Apamin has been shown to influence cardiovascular function by affecting SK channels in the heart, which play a role in regulating the pacemaker activity and contractility of the heart muscle. However, this aspect of apamin's effects is less well studied and remains an area of ongoing research.

(C.4): Safety and Toxicology: Due to its neurotoxic effects, apamin can be dangerous, especially in individuals with heightened sensitivity to bee venom. The peptide can contribute to the overall toxicity of a bee sting, leading to symptoms such as pain, muscle spasms and in severe cases, anaphylactic shock. However, in controlled settings, apamin is primarily used as a research tool rather than a therapeutic agent, due to the challenges in safely harnessing its effects. Apamin is a potent neurotoxin that plays a critical role in the neurotoxic effects of bee venom. Its specific inhibition of SK channels has made it a valuable tool for studying neuronal excitability and its implications in various neurological conditions. While it holds potential for therapeutic applications, particularly in the field of neurodegenerative diseases, its toxicity poses significant challenges that must be carefully managed in any clinical context (Dreyer & Penner, 1987).

(D): Adolapin: Constituting about 1-2% of the venom, adolapin has anti-inflammatory and analgesic properties. It inhibits the enzyme cyclooxygenase, reducing the synthesis of prostaglandins, which are involved in the inflammatory process. Adolapin is a minor peptide component of bee venom, with concentrations typically ranging from 1-2% of the venom's dry weight. It is recognized for its anti-inflammatory and analgesic properties, making it an interesting subject of study for its potential therapeutic applications (Habermann, 1972).

(D.1): Structure and Mechanism of Action: Adolapin is a polypeptide, though its precise amino acid sequence and structure are less well-characterized compared to other bee venom peptides like melittin or apamin. The peptide's primary mode of action appears to involve the inhibition of enzymes and pathways associated with inflammation and pain (Bogdanov et al., 2008).

1. **Cyclooxygenase (COX) Inhibition**:

- Adolapin is known to inhibit the enzyme cyclooxygenase (COX), particularly COX-2, which is involved in the conversion of arachidonic acid to prostaglandins. Prostaglandins are lipid compounds that play a key role in mediating inflammation, pain and fever. By inhibiting COX activity, adolapin reduces the production of prostaglandins, leading to decreased inflammation and pain.

2. **Interaction with Opioid Receptors**:

- There is some evidence suggesting that adolapin may also interact with opioid receptors, which are part of the body's natural pain control system (Habermann, 1972). This interaction could contribute to its analgesic effects, though the exact mechanism and efficacy of this action require further study.

(D.2): Biological Effects:

1. **Anti-inflammatory Properties**:

- Due to its COX-inhibitory action, adolapin has significant anti-inflammatory effects. These effects are similar to those of nonsteroidal anti-inflammatory drugs (NSAIDs), which also target COX enzymes. This makes adolapin a candidate for potential therapeutic use in conditions characterized by excessive inflammation, such as arthritis or other inflammatory disorders.

2. **Analgesic Effects**:
   - Adolapin's ability to reduce pain is closely tied to its anti-inflammatory properties. By decreasing the production of prostaglandins, which sensitize nerve endings to pain stimuli, adolapin can help alleviate pain. Its potential interaction with opioid receptors might also contribute to its analgesic properties, making it an effective pain-relieving agent in bee venom therapy.
3. **Potential Therapeutic Applications**:
   - The dual anti-inflammatory and analgesic effects of adolapin suggest it could be useful in managing chronic inflammatory conditions and associated pain. Research into adolapin could lead to the development of new therapeutic agents that mimic its effects or enhance its activity.
4. **Immune Modulation**:
   - Some studies indicate that adolapin may also have immunomodulatory effects, potentially influencing the activity of immune cells and the overall immune response. This aspect of adolapin's action is still under investigation but could have implications for treating autoimmune diseases or allergies.

(D.3): Safety and Toxicology: As with other bee venom components, the use of adolapin for therapeutic purposes must be carefully controlled due to the potential for allergic reactions and toxicity. In bee sting victims, adolapin contributes to the complex mixture of effects produced by venom, which can include localized pain and inflammation as well as systemic allergic responses. Adolapin is a relatively less-studied peptide in bee venom, but its significant anti-inflammatory and analgesic properties make it an intriguing candidate for therapeutic development. Its ability to inhibit COX enzymes and potentially interact with opioid receptors places it within the broader context of pain and inflammation management. Continued research into adolapin could unlock new avenues for treating chronic pain and inflammatory diseases (Lee et al., 2014).

(E): Mast Cell Degranulating (MCD) Peptide: This peptide induces the release of histamine from mast cells, contributing to the inflammatory response. MCD Peptide is one of the components found in bee venom, notably from the European honeybee (*Apis mellifera*). Though it constitutes a relatively small percentage of the venom's total composition, its biological effects are significant, particularly about the immune system (Sobotka et al., 1976).

(E.1): Structure and Mechanism of Action: MCD peptide is a small, cationic peptide, consisting of 22 amino acids. Its structure allows it to interact with cellular membranes and receptors, particularly those on mast cells, which are critical players in the immune response (Figure 7).

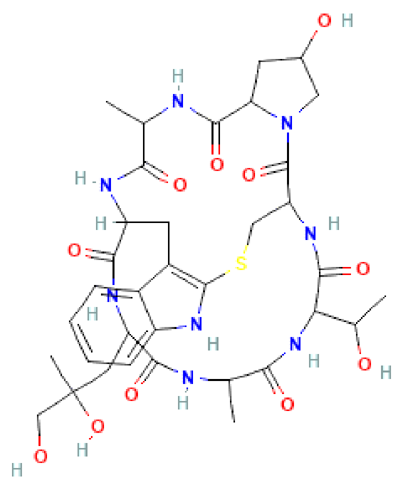

Figure 7: Structure of MCD peptide ($C_{35}H_{48}N_8O_{11}S$), Source: PubChem (4752).

1. **Mast Cell Activation**:
   - The primary action of MCD peptide is the stimulation of mast cells, a type of immune cell found in various tissues. Mast cells are packed with granules containing histamine, cytokines and other mediators of inflammation. MCD peptide binds to specific receptors on the surface of mast cells, triggering a process known as degranulation.
   - **Degranulation**: Upon activation by MCD peptide, mast cells release their granule contents into the surrounding tissue. This release includes histamine, which is a key mediator of allergic reactions, leading to increased vascular permeability, vasodilation and smooth muscle contraction. The result is the classic symptoms of inflammation, such as redness, swelling and pain.
2. **Synergistic Effects**: MCD peptide often works in conjunction with other bee venom components, amplifying the overall inflammatory response. For instance, it can enhance the effects of phospholipase A2 and melittin, leading to more increased issue damage and pain.

(E.2): Biological Effects:

1. **Inflammatory Response**:
   - The release of histamine and other mediators from mast cells contributes to the local inflammatory response seen after a bee sting. This includes pain, swelling and redness, which are typical of an acute allergic reaction.
   - In individuals with hypersensitivity to bee venom, MCD peptide can contribute to systemic allergic reactions, including anaphylaxis, which is a life-threatening condition characterized by widespread inflammation, difficulty breathing and cardiovascular collapse (Metz & Maurer, 2007).
2. **Potential Therapeutic Applications**:
   - Despite its role in promoting inflammation, the mechanisms by which MCD peptide induces mast cell degranulation have been studied for potential therapeutic applications. For example, controlled activation of mast cells could be useful in vaccine delivery, where a strong immune response is desirable.
   - Additionally, understanding the action of the MCD peptide can aid in developing treatments for allergic conditions by identifying ways to block or modulate mast cell activation.
3. **Research Tool**: MCD peptide is used in research to study mast cell behavior and the role of these cells in various allergic and inflammatory diseases. By understanding how MCD peptide induces degranulation, researchers can explore new ways to manage conditions like asthma, allergic rhinitis and chronic urticaria.

(E.3): Safety and Toxicology: MCD peptide's ability to induce mast cell degranulation makes it a potent component of bee venom. While this is beneficial for the bee as a defense mechanism, in humans, it can lead to severe allergic reactions. Individuals who are allergic to bee stings may experience heightened sensitivity to MCD peptide, contributing to the risk of anaphylaxis. Consequently, understanding and mitigating the effects of MCD peptide is important in both clinical and emergency settings.

(F): Secapin: Another peptide with potential neurotoxic and antimicrobial properties, though it is less well studied compared to others. Secapin is one of the lesser-known peptides found in bee venom, specifically from the European honeybee (*Apis mellifera*). Although it is not as extensively studied as other venom components like melittin or apamin, secapin has garnered interest due to its potential biological effects, particularly its antimicrobial and immunomodulatory properties.

(F.1): Structure and Characteristics: Secapin is a peptide consisting of about 25 amino acids. It is characterized by its cationic (positively charged) nature, which is common among antimicrobial peptides. This charge allows secapin to interact with and disrupt microbial cell membranes, contributing to its potential as an antimicrobial agent (Figure 8).

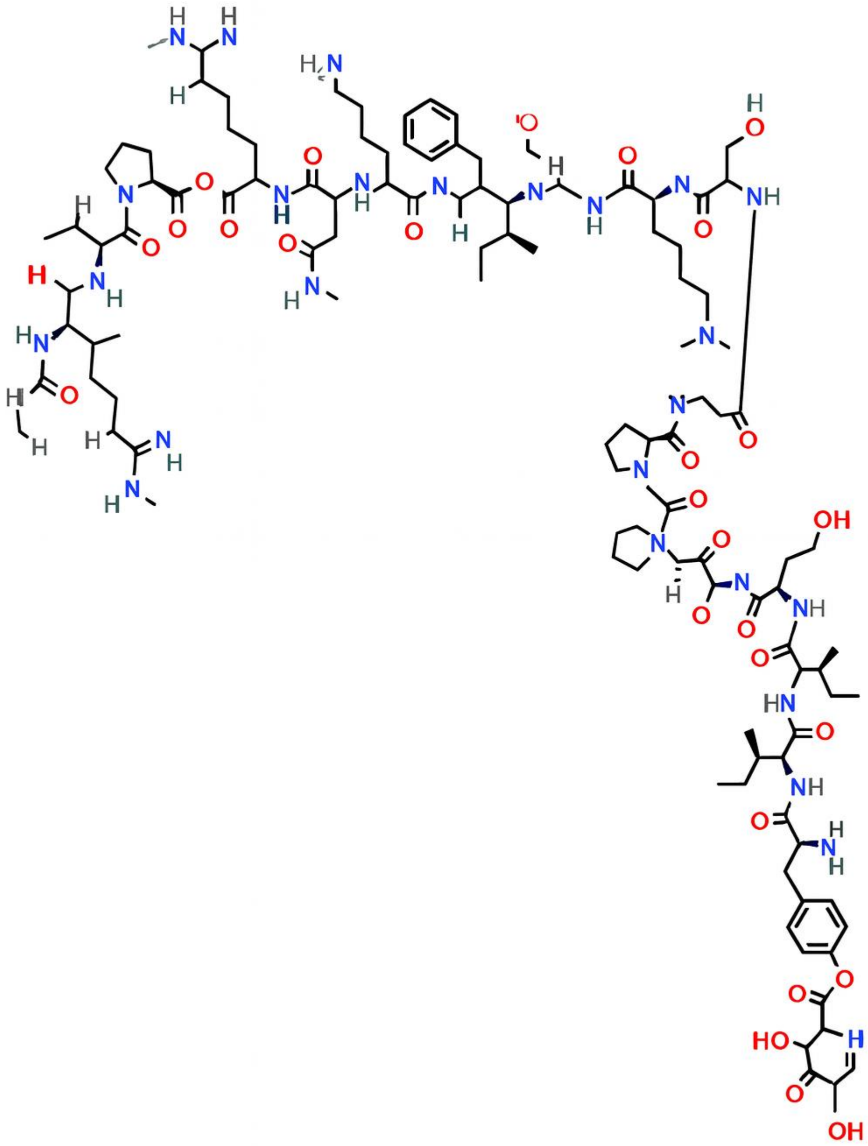

Figure 8: Structure of Secapin ($C_{131}H_{213}N_{37}O_{31}S_2$), Source: PubChem (16132134).

(F.2): Biological Effects:

1. **Antimicrobial Activity**:
   - Secapin has demonstrated antimicrobial properties against a range of pathogens, including bacteria and fungi. Like many antimicrobial peptides, secapin likely exerts its effects by binding to the negatively charged components of microbial membranes, leading to membrane disruption, leakage of cellular contents and ultimately, cell death.
   - The exact spectrum of secapin's antimicrobial activity is still being researched, but it shows promise as a natural antimicrobial agent that could be developed for therapeutic use (Mizutani & Murata, 2004).
2. **Immunomodulatory Effects**:
   - In addition to its antimicrobial properties, secapin may have immunomodulatory effects, influencing the activity of immune cells such as macrophages and lymphocytes. These effects could be beneficial in modulating immune responses, potentially offering therapeutic applications in conditions where immune regulation is necessary.
   - Secapin's role in bee venom suggests that it may also contribute to the venom's overall ability to modulate host immune responses, helping the bee defend itself against predators and pathogens.
3. **Potential Therapeutic Applications**: The combination of antimicrobial and

immunomodulatory effects makes secapin a candidate for therapeutic exploration, particularly in developing new antimicrobial agents or treatments for immune-related conditions. However, much of this potential is theoretical and more research is needed to fully understand and harness secapin's properties.

4. **Toxicity and Safety**: As a component of bee venom, secapin is part of the complex mixture of peptides and proteins that contribute to the venom's overall effects, including pain, inflammation and allergic reactions. While secapin itself may not be as toxic as other venom components, it is still part of a system that can cause significant harm, particularly in individuals who are allergic to bee stings (Hider, 1988).

(F.1): Research and Development: Research into secapin is still in its early stages compared to other bee venom components. The peptide's potential as an antimicrobial and immunomodulatory agent is being explored, with studies focusing on its mechanism of action, efficacy and possible applications in medicine. As interest in natural compounds grows, secapin may become a valuable target for developing new therapeutic agents. Secapin is a promising yet understudied component of bee venom, with potential applications in antimicrobial therapy and immune modulation. While its role in bee venom is not as well understood as that of other peptides, its biological effects make it a candidate for further research and development. As studies progress, secapin could contribute to new strategies for combating infections and regulating immune responses (Gajski et al., 2011).

2. Enzymes:

- **Hyaluronidase**: This enzyme, making up about 1-3% of the venom, degrades hyaluronic acid in connective tissues, increasing the spread of venom and facilitating the penetration of other components into tissues (Tu, 1981). Hyaluronidase is an enzyme found in bee venom, as well as in the venom of other insects, snakes and certain pathogenic bacteria. In bee venom, it plays a crucial role in facilitating the spread of venom components through tissues, earning it the nickname "spreading factor."

A. Structure and Function: Hyaluronidase is an enzyme that specifically targets hyaluronic acid, a major component of the extracellular matrix in connective tissues. Hyaluronic acid acts as a lubricant and shock absorber in joints and it contributes to tissue hydration and elasticity (Smith & Schmidt, 1996).

1. **Enzymatic Activity**:

- Hyaluronidase catalyzes the hydrolysis of hyaluronic acid, breaking it down into smaller sugar molecules. This degradation reduces the viscosity of the extracellular matrix, effectively loosening the tissue structure.
- By breaking down hyaluronic acid, hyaluronidase increases the permeability of tissues, allowing other venom components to diffuse more easily and penetrate deeper into the affected area. This enhances the venom's overall potency and facilitates the spread of other toxic and inflammatory agents, such as melittin and phospholipase A2.

B. Biological Effects:

1. **Facilitation of Venom Spread**: The primary role of hyaluronidase in bee venom is to assist in the rapid dissemination of the venom's active components throughout the stung tissue. This leads to a more widespread and intense inflammatory response, as the venom can affect a larger area more quickly.
2. **Contribution to Pain and Inflammation**: By breaking down hyaluronic acid and compromising the integrity of the extracellular matrix, hyaluronidase indirectly contributes to the pain, swelling and redness associated with a bee sting. The increased tissue permeability also facilitates the entry of immune cells,

which can exacerbate the inflammatory response.

3. **Allergenic Properties**: Hyaluronidase is also known to be an allergen in bee venom, contributing to allergic reactions in some individuals. Sensitization to hyaluronidase can lead to more severe reactions upon subsequent stings, including anaphylaxis, a life-threatening systemic allergic response.
4. **Therapeutic Uses**: Despite its role in bee venom, hyaluronidase has therapeutic applications in medicine. It is used clinically to enhance the absorption and dispersion of injected drugs, as in the case of subcutaneous fluid administration and some regional anesthetic techniques. By breaking down hyaluronic acid, hyaluronidase facilitates the diffusion of fluids and drugs through tissues, making it a valuable tool in certain medical procedures.
5. **Research Tool**: In research, hyaluronidase is used to study tissue permeability and the role of the extracellular matrix in various physiological and pathological processes. Its ability to modulate tissue structure makes it useful for experiments involving drug delivery, tissue engineering and cancer metastasis studies.

C. Safety and Toxicology: While hyaluronidase's enzymatic activity is beneficial in medical applications, its role in venom makes it a contributor to the harmful effects of bee stings. Individuals with bee venom allergies may have heightened sensitivity to hyaluronidase, leading to severe allergic reactions. In clinical use, hyaluronidase is generally well-tolerated, but it should be used with caution in patients with known allergies to bee venom or hyaluronidase itself. Hyaluronidase is a critical component of bee venom that facilitates the spread of venom through tissues by breaking down hyaluronic acid in the extracellular matrix. While it contributes to the pain and inflammation of a bee sting, it also has important medical applications, particularly in enhancing drug delivery and tissue permeability. As both a venom factor and a therapeutic enzyme, hyaluronidase exemplifies the dual nature of many biological molecules, with roles in both harm and healing (Cohen, 2009).

- **Acid Phosphatase**: This enzyme hydrolyzes phosphate esters and is involved in the mobilization of phosphorus within cells. Acid phosphatase is an enzyme found in various biological tissues, including bee venom. In the context of bee venom, acid phosphatase plays a role in the overall toxic and inflammatory effects of the venom, contributing to its complex impact on the human body (Schmidt, 1995).

A. Structure and Function: Acid phosphatase is a type of hydrolase enzyme that catalyzes the hydrolysis of phosphate esters under acidic conditions, releasing inorganic phosphate. This enzyme is found in many organisms, where it is involved in phosphate metabolism and is particularly active in lysosomes, the cellular organelles responsible for breaking down waste materials (Morton, 1958).

1. **Enzymatic Activity**: Acid phosphatase in bee venom works by dephosphorylating molecules, which can disrupt cellular processes. The enzyme's activity is optimal in acidic environments, which are often found in sites of inflammation and tissue injury, such as those caused by a bee sting.

B. Biological Effects in Bee Venom:

1. **Inflammatory Response**: Acid phosphatase contributes to the inflammation caused by bee venom. By releasing phosphate from various substrates, it may alter the local environment of the sting site, affecting cellular signaling pathways and exacerbating the inflammatory response. The enzyme’s action can lead to increased vascular permeability, allowing other venom components to spread more effectively and intensifying the local reaction, including pain, swelling and redness.

2. **Cellular Disruption**: The dephosphorylation of proteins and other molecules by acid phosphatase can disrupt normal cellular functions, leading to cell damage or death. This contributes to the overall cytotoxicity of bee venom and plays a role in its ability to disable predators or competitors.
3. **Role in Immune Modulation**: There is evidence that acid phosphatase may interact with immune cells, potentially modulating the immune response. For example, the enzyme might influence the activity of macrophages or other immune cells, though the exact mechanisms and implications of this interaction in the context of bee venom are not fully understood.

C. Therapeutic and Research Implications

1. **Biomarker in Medicine**: Acid phosphatase has clinical significance beyond its role in bee venom. For example, the prostate-specific isoform of acid phosphatase (prostatic acid phosphatase) is used as a biomarker in diagnosing and monitoring prostate cancer. Understanding the enzyme's function and mechanisms in different contexts can lead to broader insights into its role in health and disease.
2. **Potential Therapeutic Target**: Given its role in inflammation, acid phosphatase could be a target for therapeutic interventions aimed at reducing the harmful effects of bee venom or other inflammatory conditions. However, this is still largely theoretical and more research is needed to explore these possibilities.

D. Safety and Toxicology: In the context of bee stings, acid phosphatase is one of several enzymes that contribute to the venom's toxic effects. While not as well-known as melittin or phospholipase A2, it plays a supporting role in the venom's overall impact on the body. Its contribution to inflammation and cellular disruption can make bee stings particularly painful and harmful, especially in individuals who are allergic or sensitive to bee venom. Acid phosphatase in bee venom is an enzyme that contributes to the venom's ability to induce inflammation and cellular damage. By dephosphorylating molecules at the site of the sting, it plays a role in the spread and severity of the venom's effects. While it is a minor component compared to other more prominent venom constituents, acid phosphatase adds to the complexity and potency of bee venom. Its broader significance in medicine and biology as a biomarker and potential therapeutic target highlights its diverse roles across different contexts (Johansson et al., 1995).

3. Amines and Other Small Molecules:

In addition to proteins and peptides, bee venom contains various amines and other small molecules that contribute to its pharmacological effects. These components play crucial roles in the immediate response to a bee sting, particularly in inducing pain, inflammation and allergic reactions (Kettner, 1983).

Amines in Bee Venom:

1. **Histamine**:
   - Function: Histamine is a well-known biogenic amine that plays a critical role in allergic reactions and inflammation. It is released by mast cells and basophils in response to immune triggers.
   - Effects: In bee venom, histamine contributes to the immediate pain, itching and redness that occur after a sting. It increases vascular permeability, leading to swelling (edema) and promoting the infiltration of immune cells into the affected tissue. Histamine is also responsible for the sensation of itchiness often associated with insect stings.
2. **Dopamine**:
   - Function: Dopamine is another amine found in bee venom, although it is more commonly known as a neurotransmitter in the brain.
   - Effects: In the context of bee venom, dopamine may contribute to the regulation of vascular tone and local blood flow, possibly exacerbating the inflammatory

response. Its precise role in venom is less understood compared to its central nervous system functions.

3. **Norepinephrine**:
   - Function: Norepinephrine, like dopamine, is primarily known as a neurotransmitter, but it also plays a role in the venom's effects.
   - Effects: Norepinephrine can cause vasoconstriction, which might counteract some of the vasodilatory effects of histamine. This dual action helps modulate the local blood flow and inflammatory response at the sting site.
4. **Serotonin (5-hydroxytryptamine, 5-HT)**:
   - Function: Serotonin is a key neurotransmitter involved in mood regulation, but in bee venom, it acts as an inflammatory mediator.
   - Effects: Serotonin enhances pain and inflammation by promoting the contraction of smooth muscles and increasing vascular permeability. It can also amplify the effects of other inflammatory mediators in the venom, contributing to the overall painful response to a sting.

4. Other Small Molecules in Bee Venom:

1. **Phospholipids**:
   - Function: Phospholipids are part of the cell membrane components disrupted by phospholipase A2, a major enzyme in bee venom.
   - Effects: When phospholipase A2 breaks down phospholipids, it releases arachidonic acid, which is a precursor to various inflammatory mediators like prostaglandins and leukotrienes. This breakdown exacerbates inflammation and pain.
2. **Free Amino Acids**:
   - Function: Bee venom contains free amino acids, which may serve various roles, including buffering the venom's pH and contributing to its overall chemical profile.
   - Effects: The presence of these amino acids can modulate the venom's stability and effectiveness, although their specific roles are not as prominent as those of other components.
3. **Sugars**:
   - Function: Bee venom contains small amounts of sugars, which may be involved in energy metabolism or other cellular processes within the venom gland.
   - Effects: The sugars in venom do not have direct pharmacological effects, but they may play a role in the overall composition and viscosity of the venom.
4. **Acetylcholine**:
   - Function: Acetylcholine is a neurotransmitter involved in muscle activation and various autonomic nervous system functions.
   - Effects: In bee venom, acetylcholine can stimulate pain receptors and contribute to the sensation of pain at the sting site. It may also influence the contraction of smooth muscles in blood vessels and other tissues, impacting the local response to venom (Tu, 1981).

The amines and other small molecules in bee venom are crucial for the immediate effects experienced after a sting, including pain, itching and inflammation. These components work synergistically with the larger proteins and peptides in the venom to create a complex and potent defense mechanism for the bee. Understanding these molecules not only helps in managing bee stings but also provides insights into potential therapeutic applications and the broader pharmacological effects of bee venom (Weintraub & Moring, 1975).

Therapeutic Applications: Bee venom has been explored for various therapeutic applications, including treatment for arthritis, multiple sclerosis and other inflammatory or immune-related conditions. Its components have potential antibacterial, antiviral and anticancer properties, but further research is required to fully understand and harness these effects (Son et al., 2007, & Ali, 2012). Bee venom, also known as apitoxin, has been used in traditional medicine for centuries and is now being studied for various therapeutic applications. Its complex mixture of peptides, enzymes and small molecules has shown potential in treating a range of medical conditions, particularly those involving pain, inflammation and immune modulation (Wehbe et al., 2019).

1. **Rheumatoid Arthritis and Inflammatory Conditions**:

- Mechanism: Bee venom contains compounds like melittin, which has potent anti-inflammatory effects by inhibiting the enzyme phospholipase A2 and blocking the production of pro-inflammatory cytokines.
- Applications: In rheumatoid arthritis (RA), bee venom therapy (BVT) has been used to reduce pain and inflammation. Clinical studies have shown that BVT can decrease the severity of symptoms in patients with RA, potentially reducing the need for conventional anti-inflammatory drugs.
- Evidence: A study published in the *Journal of Ethnopharmacology* found that bee venom acupuncture significantly reduced pain and improved joint function in RA patients (Son et al., 2007).

2. **Neurological Disorders**:

- Mechanism: Bee venom and its components, such as apamin, have neuroprotective properties. Apamin blocks specific potassium channels in the brain, which can enhance neuronal survival and function.
- Applications: Bee venom has been investigated as a treatment for neurological conditions like Parkinson's disease, multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Its potential to modulate the immune system and protect neurons makes it a candidate for these diseases.
- Evidence: Research in animal models of Parkinson's disease has shown that bee venom can reduce neuroinflammation and protect dopaminergic neurons, which are critical in the disease's pathology.

3. **Pain Management**:

- Mechanism: Bee venom components, particularly melittin and adolapin, have analgesic properties. Melittin can modulate pain signaling pathways, while adolapin inhibits cyclooxygenase, an enzyme involved in pain and inflammation.
- Applications: Bee venom therapy has been used to manage chronic pain conditions, including back pain, osteoarthritis and fibromyalgia. By reducing inflammation and modulating pain perception, bee venom can offer relief where conventional painkillers may be insufficient.
- Evidence: Studies have demonstrated that bee venom acupuncture is effective in reducing pain intensity in patients with chronic low back pain, with effects comparable to standard pain management techniques.

4. **Cancer Treatment**:

- Mechanism: Bee venom, particularly melittin, has shown anticancer properties. Melittin can induce apoptosis (programmed cell death) in cancer cells and disrupt the formation of new blood vessels (angiogenesis) that tumors need to grow.
- Applications: Bee venom is being researched as an adjunctive treatment in cancer therapy. It has shown promise in preclinical studies against various types of cancer, including breast cancer, prostate cancer and melanoma.
- Evidence: In vitro studies have shown that melittin can inhibit the growth of cancer cells and enhance the efficacy of chemotherapy drugs. However, more clinical research is needed to establish its role in cancer treatment.

5. **Immunotherapy and Allergy Treatment**:

- Mechanism: Bee venom can modulate the immune response, making it a potential tool in immunotherapy. By exposing patients to controlled doses of bee venom, the immune system can be desensitized, reducing the severity of allergic reactions to bee stings.
- Applications: Bee venom immunotherapy (BVIT) is used to treat individuals who are allergic to bee stings. This therapy gradually increases the patient's tolerance

to bee venom, reducing the risk of anaphylaxis upon subsequent stings.
- Evidence: BVIT has been shown to be effective in about 80-90% of patients with bee sting allergies, significantly lowering the risk of severe allergic reactions.

6. **Skin Disorders**:
- Mechanism: Bee venom has anti-inflammatory, antimicrobial and collagen-stimulating properties, making it useful in treating various skin conditions.
- Applications: It is used in dermatology for conditions like acne, eczema and psoriasis. Bee venom is also incorporated into cosmetic products for its potential anti-aging effects, as it can stimulate collagen production and improve skin elasticity.
- Evidence: Clinical trials have shown that topical bee venom can reduce acne lesions and improve skin hydration and elasticity, leading to its inclusion in various skincare products.

7. **Antimicrobial Activity**:
- Mechanism: Bee venom contains antimicrobial peptides, such as melittin and secapin, which can disrupt bacterial cell membranes and inhibit the growth of various pathogens.
- Applications: Bee venom is being explored as a natural antimicrobial agent to treat infections, particularly those resistant to conventional antibiotics. Its potential to combat bacterial, fungal and even viral infections is under investigation.
- Evidence: Studies have demonstrated the effectiveness of bee venom components against antibiotic-resistant strains of bacteria, suggesting it could be a valuable tool in the fight against antimicrobial resistance.

8. **Wound Healing**:
- Mechanism: The anti-inflammatory and antimicrobial properties of bee venom, along with its ability to promote tissue regeneration, make it beneficial in wound healing.
- Applications: Bee venom has been used in the treatment of chronic wounds, such as diabetic ulcers and pressure sores. Its components help reduce infection and inflammation and promote faster healing.
- Evidence: Research has shown that topical application of bee venom can accelerate wound healing by reducing bacterial load and promoting the formation of new tissue.

Bee venom holds significant promise in a wide range of therapeutic applications, from managing chronic pain and inflammatory diseases to offering potential treatments for neurological disorders, cancer and skin conditions. While its therapeutic use is supported by both traditional practices and modern research, it is important to approach bee venom therapy with caution due to the risk of allergic reactions. Continued research and clinical trials are essential to fully understand its benefits and to develop safe, effective treatments **(**Hwang et al., 2013; & Maitip et al., 2021).

CONCLUSION

Bee venom, a complex mixture of peptides, enzymes and small molecules, has garnered significant attention for its diverse therapeutic potential. From its traditional use in treating ailments like arthritis to its modern applications in neurological disorders, pain management and even cancer therapy. Bee venom demonstrates a wide range of pharmacological effects. The anti-inflammatory, immunomodulatory and antimicrobial properties of its components, such as melittin, apamin and phospholipase A2, make it a versatile tool in both conventional and alternative medicine. However, the therapeutic applications of bee venom are promising. They must be approached with caution due to the risk of allergic reactions, including anaphylaxis, in sensitive individuals. Continued research and clinical trials are necessary to fully understand the benefits and limitations of bee venom therapy, ensuring its safe and effective use in various medical contexts. As our understanding of bee venom deepens, it may lead to the development of new treatments that harness its powerful bioactive compounds, offering novel solutions to some of today's most challenging health issues.

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REFERENCES

Adelman, J. P., Maylie, J., & Sah, P. (2012). "Small-conductance Ca2+-activated K+ channels: Form and function." *Annual Review of Physiology*, *74*, 245-269.

Ali, M. A. (2012). Studies on Bee Venom and Its Medical Uses. *International Journal of Advancements in Research & Technology*, *1*(2), 69–85.

Annand, R. R., Kontoyianni, M., Penzotti, J., Dudler, T., Lybrand, T. P., & Gelb, M. H. (1996). Active site of bee venom phospholipase A2: the role of histidine-34, aspartate-64 and tyrosine-87. *Biochemistry, 35 14*, 4591-601.

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). "Honey for Nutrition and Health: A Review." *American Journal of Clinical Nutrition*, *87*(1), 153S-157S.

Bon, C. (1997). "Phospholipases A2 as venom components and tools in pharmacology and neurobiology." *Toxicon*, *34*(9), 1167-1176.

Cohen, S. (2009). "Hyaluronidase." *Dermatologic Surgery*, *35*(4), 545-549.

Dotimas, E. M., & Hider, R. C. (1987). "Honeybee venom." *Bee World*, *68*(2), 51-70.

Dreyer, F., & Penner, R. (1987). "Apamin blocks a calcium-activated potassium conductance in rat skeletal muscle fibres." *Pflügers Archiv*, *409*(6), 548-551.

El Mehdi, I., Falcão, S. I., Boujraf, S., Mustapha, H., Campos, M. G., & Vilas-Boas, M. (2022). Analytical methods for honeybee venom characterization. *Journal of advanced pharmaceutical technology & research*, *13*(3), 154–160. https://doi.org/10.4103/japtr.japtr_166_21

Gajski, G., Garaj-Vrhovac, V., & Trosić, I. (2011). "Bee venom and its components as potentially new therapeutic modalities." *Cytotechnology*, *63*(6), 553-565.

Gajski, G., Leonova, E., & Sjakste, N. (2024). Bee Venom: Composition and Anticancer Properties. *Toxins*, *16*(3), 117. https://doi.org/10.3390/toxins16030117

Habermann, E. (1972). "Bee and wasp venoms." *Science*, *177*(4046), 314-322.

Hider, R. C. (1988). "Honeybee venom: A rich source of pharmacologically active peptides." *Endeavour*, *12*(2), 60-65.

Hider, R. C., & Ewing, W. R. (1982). "The primary structure of the mast cell degranulating peptide (MCDP) from bee venom." *Toxicon*, *20*(1), 173-178.

Hwang, J. T., Park, I. J., & Shin, J. I. (2013). "Bee venom liposome reduces pain in a rat model of monosodium iodoacetate-induced knee osteoarthritis." *Journal of Pain Research*, *6*, 95-101.

Johansson, T., Elfsmark, L., & Edqvist, L. E. (1995). "Acid phosphatase in the context of bee venom: Comparative aspects and clinical relevance." *Acta Veterinaria Scandinavica*, *36*(1), 55-65.

Kettner, K. (1983). "Neurotransmitter amines in insect venom: An overview." *Comparative Biochemistry and Physiology*, *74*(1), 1-12.

Kini, R. M. (1997). "Phospholipase A2—a complex multifunctional protein puzzle." *Bioscience Reports*, *17*(4), 245-258.

Lee, G., & Bae, H. (2016). Bee venom phospholipase A2: Yesterday's enemy becomes today's friend. *Toxins*, *8*(2), 48.

Lee, W. R., Kim, K. H., & An, H. J. (2014). "The protective effects of bee venom against photocarcinogenesis by UVB irradiation." *Phytotherapy Research*, *28*(11), 1717-1723.

Maitip, J., Mookhploy, W., Khorndork, S., & Chantawannakul, P. (2021). Comparative Study of Antimicrobial Properties of Bee Venom Extracts and Melittins of Honey Bees. *Antibiotics*, *10*(12), 1503. https://doi.org/10.3390/antibiotics10121503

Memariani, H., Memariani, M., & Shahidi-Dadras, M. (2019). "Melittin: From honeybees to superbugs." *Applied Microbiology and Biotechnology*, *103*(8), 3265-3276.

Metz, M., & Maurer, M. (2007). "Mast cells—key effector cells in immune responses." *Trends in Immunology*, *28*(5), 234-241.

Mizutani, H., & Murata, M. (2004). "Antimicrobial peptides from the honeybee." *Biopolymers*, *74*(1-2), 118-122.

Morton, R. K. (1958). "The purification and properties of acid phosphatase." *The Biochemical Journal*, *69*(3), 444-458.

Oršolić, N. (2012). Bee venom in cancer therapy. *Cancer Metastasis Reviews*, *31*(1-2), 173-194.

Pandey, P., Khan, F., Khan, M. A., Kumar, R., & Upadhyay, T. K. (2023). An Updated Review Summarizing the Anticancer Efficacy of Melittin from Bee Venom in Several Models of Human Cancers. *Nutrients*, *15*(14), 3111. https://doi.org/10.3390/nu15143111

Raghuraman, H., & Chattopadhyay, A. (2007). "Melittin: A membrane-active peptide with diverse functions." *Bioscience Reports*, *27*(4-5), 189-223.

Schmidt, J. O. (1995). "Toxicology of venoms from the honeybee genus Apis." *Toxicon*, *33*(4), 397-409.

Smith, M. L., & Schmidt, J. O. (1996). "Role of venom and venom allergens in the allergic response to stings." *The Journal of Allergy and Clinical Immunology*, *97*(1), 156-160.

Sobotka, A. K., Franklin, R. M., & Adkinson, N. F. (1976). "Immediate Hypersensitivity Responses to a Peptide from Bee Venom." *Journal of Allergy and Clinical Immunology*, *58*(6), 567-572.

Son, D. J., Lee, J. W., Lee, Y. H., Song, H. S., Lee, C. K., & Hong, J. T. (2007). Therapeutic application of anti-arthritis, pain-releasing and anticancer effects of bee venom and its constituent compounds. *Pharmacology & Therapeutics*, *115*(2), 246-270.

Stocker, M. (2004). "Ca2+-activated K+ channels: Molecular determinants and function of the SK family." *Nature Reviews Neuroscience*, *5*(10), 758-770.

Tu, A. T. (1981). "Insect venoms: Biochemistry and pharmacology." *Toxicology*, *16*(1), 1-24.

Wehbe, R., Frangieh, J., & Rima, M., (2019). "Bee venom: Overview of main compounds and bioactivities for therapeutic interests." *Molecules*, *24*(16), 2997.

Weintraub, S. T., & Moring, J. (1975). "Free amino acids in bee venom: Qualitative and quantitative analysis." *Journal of Chromatography A*, *108*(1), 167-175.

Xu, X., Han, X., & Qiu, Y. (2010). "The structure, function and potential application of bee venom phospholipase A2." *Molecules*, *15*(8), 4935-4951.

Zhang, S., Liu, Y., Ye, Y., Wang, X., & Lin, L. (2018). The anticancer effect of bee venom: A systematic review and meta-analysis. *Journal of Ethnopharmacology*, *211*, 108-123.