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Review Article

BEES AND WASPS VENOM TOXINS, ITS IMMUNE-ALLERGIC RESPONSES, DIAGNOSIS AND THERAPEUTICS

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ABSTRACT

Present article explains insect toxins, its immune allergic, pharmaceutical and therapeutic effects. Insect venom glands generate enzymatic and non-enzymatic toxins and are inflicted by the stings. Insect's envenomation are highly painful, inflamed and life-threatening. It causes breathing difficulties, bronchospasm, hypotension and arrhythmia, cardiopulmonary problems, and imposes allergic reactions. Wasp venom toxins generate strong T-cell responses in hypersensitivity patients and stimulate the production of IgE antibody molecules. Massive envenomations causes the death of victims due to the toxic effects of the venom toxins if clinical treatment is delayed. This article also emphasizes the role of natural and recombinant toxins for the development of highly sensitive immune-assays for diagnosis of allergen-specific tolerance, its early and delayed effects in patients to avoid fatal anaphylactic reactions. It also directs about the essentiality of immune diagnostics, vaccines and antiserum therapy in high population density regions where incidences of wasp and bee envenomations are more frequently occur. Venom immunotherapy can restore normal immunity against venom allergens and may also provide lifetime tolerance against venoms. This article highlights the major effects of insect venom allergens, its diagnosis and venom immunotherapy.

Keywords: Wasps and honeybee, Allergens, Immune-hypersensitivity, Venom immunotherapy

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INTRODUCTION

Hymenoptera is an order of the class Insecta comprises important families, i.e. Apidae, Vespidae, and Myrmicidae. These represent venomous insects, including *honeybees*, *bumblebees*, paper wasps, hornets, ants. These insects possess specialized stinging apparatus that is used to inject venom into prey's or intruder's body. Stinging by wasps, bees and hornets could be life-threatening for people sensitive to the venom. Members of family Apidae and Vespidae inflict stings very fast and results in allergic reactions and display fast to delayed hypersensitivity reactions. Bumblebees are allergic, but relatively less aggressive than wasps and honey bees and largely assist in plant pollination [1]. Fire ants are representative of the family Myrmicidae and cause local inflammation and its venom is less sensitive than other bees and wasps [2]. Hymenopteran stinging evokes hypersensitivity that causes a number of deaths in the world than any other envenomations [3]. Envenomation by hymenopterans imposes anaphylactic effects such as generalized urticaria, bronchospasm, hypotension, cardiovascular collapse and loss of consciousness.

Venom toxins also impose systemic toxic reactions such as edema, vomits, diarrhea, headache, hypotension, seizures, altered mental status and any unusual reactions such as cardiac ischemia and encephalomyelitis that usually lasts for 1-2 d [4]. Anaphylaxis is life-threatening as imposing cardiopulmonary collapse and generates breathing difficulties, bronchospasm, hypotension and arrhythmia [5]. In extreme cases, anaphylaxis appeared as systemic symptoms involving the gastrointestinal tract, genitourinary tract, or the nervous system. It generates a severe systemic reaction in children as they display mild eosinophilia, female sex and concomitant atopic diseases [6]. Severity of allergy depends on immune tolerance as it is seen in beekeepers, but in normal people, it shows an increased risk of sting anaphylaxis due to intense allergen exposure, allergy and low-grade systemic reactions [7] (table 1). But most deaths occur due to the occurrence of immediate hypersensitivity reactions and anaphylaxis. In the present review, pharmacological and therapeutic effects of major insect venom toxins have been broadly explained for management after stings.

Source of information

For writing this comprehensive research review on hymenopteran insect toxins/allergens, various databases were searched. For the collection of relevant information specific terms such as medical subject headings (MeSH) and key text words, such as "venom allergens", "biological and pharmaceutical effects", venom immunotherapy drug development" published till 2020 were used in MEDLINE. Most especially for retrieving all articles pertaining to the use of VIT for insect venom allergy, electronic bibliographic databases were searched and abstracts of published studies with relevant information on the venom toxins/allergens were collected. Furthermore, additional references were included through searching the references cited by the studies done on the present topic. Relevant terms were used individually and in combination to ensure an extensive literature search. For updating the information about a subject and incorporation of recent knowledge, relevant research articles, books, conference proceedings' and public health organization survey reports were selected and collated based on the broader objective of the review. This was achieved by searching databases, including SCOPUS, Web of Science, and EMBASE, Pubmed, Swissprot, Google searches. From this common methodology, discoveries and findings were identified and summarized in this final review.

Phyiological effects

Physiologically animal toxins are highly active natural substances which bind to various channels and break the normal barrier to the free movement of molecules across cell membranes. Venom toxins cause enormous hemolysis of RBCs and damage nerve cells. These specifically act upon neurons, nicotinic acetylcholine receptors and neuromuscular junctions. Hymenopteran venom toxins cause hypotensive effects, while a non-enzymatic neurotoxic peptide fraction produced the hypertensive effects Phospholipase A, an enzymatic toxin, severely acts upon motor nerve terminals and muscle cells. It damages skeletal muscles and inhibits cell regeneration. Bee venom contains melittin (membranous toxin), albumin (neurotoxin), tertiapin, MCD-peptide (mast cell degranulating peptide), acidic phosphatase, as well as phospholipase A2 and hyaluronidase, both possessing allergenic properties (table 1). Melatonin is an enzymatic toxin that hydrolyzes membrane phospholipids and form channels through which small molecules may pass. Protease inhibitors found in venom bind to protease

enzymes and prevent their actions. These inhibit fibrin activity in arthritic joints and induce chronic arthritis.

Various venom toxins isolated from bees, wasps and scorpion induces immuno-modulatory, cardio-respiratory, analgesic and homeopathic effects. These venom toxins cause sudden inflammation in body cells with severe pain and do massive inhibition of axonal transmission in neurons. Toxins also change the orientation and affinity of ion binding sites and change ion permeability mediated by the nicotinic ACH receptors. Different animal groups have different channel inhibitors. Some toxins are inhibitors of metabolic enzymes and have hydrophobic pockets in their secondary structure by which the venom bind at specific substrate binding sites. The toxicity of venom depends upon the sequence of amino acid residues present in the active site regions, topological folding, hydrophobic pockets and binding affinity. The mode of action of animal toxins and their physiological consequences varies greatly according to the structural variability in the active site region (table 1).

Venoms also cause acute and chronic inflammatory responses in laboratory animals. These affect ATP as driven Na^+-K^+ATPase pumps, that plays a key role in maintaining cell volume and intracellular ionic composition, specially $Na^+$and K^+ gradients is also affected. This pump actively transports ions across the cell membrane, helps in the excitation of nerves, and does phosphorylation and dephosphorylation in muscle cells. In this mechanism some transmembrane proteins/enzymes utilize the energy stored in molecules of ATP to move K^+ into the neuron. Na^+-K^+pump assists the neurons in maintain of resting potential for which pump allows interior negative charge and exterior positive charge on neurons by pumping Na^+outside the cell and K^+inside the cell. Both $Na^+$and K^+channels are competitively blocked by these toxins, induce the release of transmitters and cause repetitive firing of the axons and cause repetitive firing of the axons. Wasp and bee envenomation causes severe pain, redness, swelling, heavy inflammation, fever, problem in breathing and severe cardiovascular problems. Reactions to Hymenoptera stings are classified into normal local reactions, large local reactions, and systemic effects. However, for therapeutic management stinger is removed out and local remedies such as ice-packs, topical steroid epinephrine and beta-mimetics, fluid resuscitation, oxygen therapy is provided to patient for prevention and treatment of an anaphylactic shock.

The hymenopteran venom consists of a mixture of biologically active substances, such as enzymes phospholipases, hyaluronidases and toxin peptides such as mellitins, apamins, mastoparans, bombolitins and low-molecular-weight compounds Biogenic amines, acetylcholine, carbohydrates, lipids, free amino acids. Predatory wasps i.e. hornets (*Vespa* and *Dolico vespula*), yellow jackets and paper wasps possess amines, small peptides and high molecular weight proteins such as enzymes, allergens and toxins [8]. Wasp venom contains mastoparan and kinins both generate toxic effect, while phospholipase A and B, hyaluronidase, antigen 5, acidic phosphatase, and alkaline phosphatase cause allergenic reactions [9, 10]. Three major allergenic proteins, such as antigen 5, phospholipase, and hyaluronidase are found in venom of vespula and hornets. While the *Dolicho vespula* venoms are also allergenic in nature [11, 12]. Allergen antigen 5, a hypoallergenic variance is also detected in *Polibias cutellaris* [13] (table 1).

Venom toxins are primary actors for toxicity in animal venoms [14]. These generate severe allergic effects in victims [15]. Wasp sting shows nephrotoxicity and impose cortical necrosis (CAN) in the kidney [16], severe lung hemorrhage [17], fever [18] systemic reactions includes anaphylactic shocks [19] and genotoxic or mutagenic effects [20]. Insect venom also possesses vasoactive and thrombogenic substances cause vasospasm and coronary thrombosis [21]. Stinging by two insects of two different species show cross-reactivity and show severe allergic reaction because its venoms differs in terms of composition [22]. Bee and bumblebee venom allergen show cross-reactivity [23]. Similar cross-reactivity is also detected between the venoms of yellow jacket wasps and hornets [24]. This cross-reactivity might be due to the similarity of epitopes of antigens contained in venoms of various insect species. Most common observation about bees, they die immediately after injecting venom, while the wasp can sting several times. Bees sting a single time, but wasp inflicts venom repetitively but an allergic reaction is lower than in the bee [25].

Immune allergic responses

Wasp and bee venom is immunogenic in nature. It induces type I reaction, a most common type of immune response which generate in initial stage of allergy. The wasp venom generates immediate and delayed hypersensitivity reactions that resulted in fatal IgE-mediated anaphylactic reactions in allergic individuals [26]. Insect venoms also cause non-allergic local reactions such as pain, small demo, and redness at the site of the sting. First contact with the allergen present in the venom leads to the synthesis of specific IgE antibodies by allergen sensitized B lymphocytes during generation of humoral response. As soon as antigens interact with antibodies bound to the surface of mast cells, results in degranulation of these cells. These start the release of preformed mediators mainly histamine. Some of the other important mediators are prostaglandins, leukotrienes which are synthesized de novo. The release of these molecules accelerates permeability of capillaries, dilatation of venous vessels, and start constriction of bronchial and alimentary tract muscular membrane. It is, further, reflected by the cascade of clinical signs leading to the anaphylactic reaction. Mechanisms leading to the activation of specific IgE synthesis are complex. Synthesis of few important molecules interleukins IL-4 and IL-13 get increased and IFN-γ is synthesized at a faster rate with the surface activation of T cell markers (CD40L and CTLA-4). In anaphylaxis important role is played by enhanced synthesis of interleukins IL-4 and IL-13, as well as by the predominance of Th2 phenotype in lymphocyte population. Activation of CD40 on the surface of B lymphocyte by its ligands present on the surface of T lymphocyte (CD40L) is an interaction required for the formation of memory B cells. This reaction leads to the synthesis of antibodies against T-cell dependent antigens and inhibits the apoptosis of B lymphocytes [27].

Pathological signs and symptoms

Upon infliction, venom causes large local allergic reactions or systemic reactions [28] i.e., displayed in form of intradermal lesion an itchy wheal, up to 2 cm in diameter which depends on the individual sensitization pattern [29]. This is highly painful and surrounded by swelling of subcutaneous tissue, up to 10 cm in diameter that persists for 24 h. The main symptom of immediate type allergy is red, round spots at the site of stinging. Both toxic reactions and cytotoxic effects are generated due to the presence of specific components of the venom that also causes hemolysis and rhabdomyolysis, with subsequent, potentially lethal renal injury [30]. Mastoparans and kinins found in wasps venom and both generate toxic effects while phospholipase A and B, hyaluronidase, antigen 5, acidic phosphatase, and alkaline phosphatase generate allergic reactions. Allergic reactions are classified as local or systemic. Local allergic reaction pertains to a swelling and rash larger than 10 cm and persisting for more than 24 h. More specially, stinging within the neck and head regions are potentially most dangerous. However, in reality, the swelling resulting from neck sting is usually markedly smaller than that located within a limb. The clinical effects of a systemic allergic reaction are highly variable because of component-specific difference and its interaction.

Post stinging reactions of hymenopteran are both non-allergic and allergic character. These are categorized into four levels or grades. In the first level or stage I venom stings causes erythema, pruritis, urticaria, Angio-oedema, rhinitis, nausea, intense pain, mild to moderate local swelling with redness around the sting site, warmth or inflammation at the sting site. Level II or stage II displayed asthma, Angio-oedema, abdominal pain, redness and swelling that extends beyond the sting site, persists for a couple of days. It remains up to 5-10 d. Stage III or level III causes severe breathing or respiratory difficulties displayed by Laryngeal edema or asthma, marked hypotension, collapse, loss of consciousness, dyspnea, laryngeal stridor, dysphagia, dysarthria, dysphonia, and fear of death. Stage IV or level IV displays a decrease in arterial pressure, collapse, loss of consciousness, urinary and fecal incontinence, and cyanosis. The last stage is also specified by systemic allergic reaction

or anaphylaxis with swelling of face, throat, lips and tongue, restlessness, anxiety, sharp drop in blood pressure, fast pulse and dizziness. The patient also gripped in wheezing, dizziness, and lower blood pressure, and light-headedness, loss of consciousness, nausea or vomiting. Usually, the systemic reaction develops within 10 min of stinging [31].

Venom composition

The venom of hymenopterans (bees and wasp) are a complex mixture containing proteins, enzymes and small molecules, including some of the most dangerous allergens. It contains enzymes, non-enzymatic constituents. Hymenoptera venom quantity (w/v) differs from species to species, age of the insect. On an average the protein content per single injection of venom amounts to 50–70 µg in the bee, 4. 0–16 µg in the wasp, and 10–30 µg in the bumblebee [31]. The venom protein of the hymenopteran is categorized in 3 groups. Group A consists of enzymatic components such as hyaluronidases, phospholipases, Group B Non-enzymatic components (peptide components) such as antigen-5, mastoparans, wasp Kinase while group C represents phospholipases, hyaluronidases and mastoperans and non-peptidic components like alkaloids and polyamines. Hymenoptera venoms each contain a variety of protein allergens. Solitary and social wasp venoms include hyaluronidase, phospholipase A2, metalloendopeptidase etc. Enzymes phospholipase A1, phospholipase A2, acid phosphatase, hyaluronidase, serine protease and antigen 5 are common venom allergens of hymenopterans [32] (table 1). Many types of enzymes found in the *Polistes flavus* venom such as hydrolases (proteases, hyaluronidases, phospholipases and nucleotides) all; these enzymes are grouped into hydrolases as well as allergens [33] and toxins [34]. The most abundant proteins identified in parasitoid venom are hydrolases, such as proteases, peptides, esterases, glycosyl-hydrolase and endonucleases. Few parasitoid venom proteins functions as extracellular superoxide dismutase-3, serine protease inhibitor and calreticulin [35]. Fewer toxins possess highly stable inhibitors cysteine knot (ICK) motif 2 and 4 disulphide bonds. These were found ion channel blockers (neurotoxins) and hemolytic agents [36].

Component specific allergic reactions

(i) Hyaluronidases

Hyaluronidases are glycoproteins found in the venom of honey bees, wasps and snake venom. These are a family of enzymes that catalyze the degradation of hyaluronic acid. Hyaluronidases found in almost all venoms and are known as spreading factor. It contains N-glycans, which are responsible for allergenicity. It is a glycoside hydrolase that breaks β-14 glycosidic bonds between N-acetylglucosamine and D-glucuronic acids of the hyaluronic acids [37]. Hyaluronidases mainly hyaluronoglucosidases (EC 3.2.1.35), i.e., cleave the (1->4)-linkages between N-acetylglucosamine and glucuronate. They are allergenic factors in the bee and wasp venoms and induce severely anaphylactic and IgE-mediated reactions in mammals [38]. It initiates the release of histamine from mast cells that is responsible for the immediate type of hypersensitivity reactions [39]. The hyaluronidases in vespid venoms are similar to bee venom hyaluronidase [40]. The enzymes contain significant amounts of N-linked carbohydrate [41]. These possess three groups of hyaluronidases i. e endo-β-N-acetyl-D-hexosaminidases that hydrolyze the higher molecular weight substrates to tetrasachharides as the main end products. Second is β-endoglycoridases group that is detected in leeches and the hookworms. The third groups of the losses that act via β-elimination yielding disaccharides as the main products reported in bacterial hyaluronidases (table 1).

(ii) Phospholipases

Phospholipases are lipolytic enzymes that hydrolyze phospholipid substrates at specific ester bonds into fatty acids and other lipophilic substances. Phospholipases are widespread in nature and play very diverse roles, from aggression in snake venom to signal transduction, lipid mediator production, and metabolite digestion in humans. There are four major classes, termed A, B, C and D, which are distinguished by the type of reaction which they catalyze. Phospholipase A2 acts on the intact lecithin molecule and hydrolyzes the fatty acid esterified to the second carbon atom. The resulting products are lysolecithin and a fatty acid. Phospholipase A2 is an enzyme present in the venom of bees and viper snakes. Phospholipases are enzymes that hydrolyze the ester bonds of the phospholipids and fatty acids.

The wasp venom belongs to a different superfamily than those of bees venom phospholipases. Wasp venom phopholipases have PLA, PLB specificity and are numbered lipases. Several major glycoprotein components have been identified in the venom of bee and wasp. The most important specific major components are API m 1 (phospholipase A2) in the bee, Ves v 1 (phospholipase A1) and Ves v 5 (antigen 5) in the wasp. The phospholipids from the *Polistes* venom usually do not contain carbohydrates and have a highly homologous region of active sites. The phospholipids of the wasp venom digest the cell wall components of diacyphospholipids such as phosphotidylcholine, phosphotidylserine, phosphotidyl ethanolamine to fatty acids and lysophospholipids with PLAs. The phospholipases B is more universally digestive enzymes than PLA1 and PLA2 and this are present in the venom of yellow wasp *Polistes flavus* (table 1). The phospholipases have not only digestive enzyme, but also have hemolytic activities and cardiotoxicity [42]. The phospholipase A1 hydrolyzes the ester bonds of the phospholipids at the Sn-1 position and produce 2-alkyl-lysophospholipids and fatty acids. PLA2 from the *Polistes* venom disrupt the phospholipids packing from several types of biological membranes, leading to pores formation and cell-lysis [43].

(iii) Antigen 5

Antigen 5 is a major and most thoroughly studied of the vespid venom allergens [44-48]. Antigen 5 is the high molecular weight protein but does not contain carbohydrate [48]. Wasp antigen 5 It shows the different size in wasp species of different genera. In *Polistes flavus* venom, the antigen 5 has been made up by the 201-205 amino acids respectively with very high conserve region protein. Antigen 5 from *Dolicho vespula* and the Vespa is not always cross-react with antigen 5 from other species of hornets or *Vespula* [48]. The antigen 5 homologs from fire ants do not exhibit antigenic cross reactivity with those of vespid wasps [48]. Antigen 5 is a venom protein of hornets, yellow jackets and wasps, and it is an important allergen for insect-sensitive patients (table 1). Antigen 5s from these insects is found to have similar amino-terminal sequences for their first 20 residues, and to have similar cyanogen bromide cleavage patterns. A partial sequence homology of antigen 5s and scorpion neurotoxins is observed. Antigen 5s from these insects show varying extents of cross-reactivity when tested with mouse polyclonal antibodies by enzyme immunoassay. This protein, designated as Antigen 5 (Dol m V), has 204-205 amino acid residues and it contains 4 disulfide bonds. This protein is an allergen in man, but its biological function in hornet is not yet known.

(iv) Mastoparans

Mastoparans are low molecular and α-helical polycationic amphipathic linear tetradecapeptide amides found in venom of the wasp *Vespula lewisii* [49] (table 1). They are rich in hydrophobic leucine, isoleucine, alanine and basic residues, which maintain electrostatic interactions with the negatively charged phospholipids head groups of the biological membranes [50]. Vespid venoms are represented by the mastoparans; it involves a histamine-releasing principle. Vespid venoms are represented by the mastoparans, it involves a histamine-releasing principle. Mastoparans are antimicrobial in nature, causes mast cell degranulation and show hemolytic activities [50]. The amphiphilic nature of these peptides makes them interact with different types of cell membranes through different mechanisms, depending on their primary sequences. Non-lytic mastoparans may present the ability to interact with different types of cell strains, causing minimal membrane reorganization, but interact with cell membrane receptors, especially those coupled to G-proteins. The high affinity of these receptors makes the mastoparans molecules very attractive lead compounds to develop a new generation of drugs to modulate the activity of G-proteins-coupled cell signaling system. Mastoparan stimulates insulin secretion from βTC3 and INS-1 cells [50] (table 1).

Mastoparan peptide found inserted into the membrane bilayer and thus interacts directly with G-proteins on the cytoplasmic face attacking the transmembrane signaling and induced by an increase in cytoplasmic Ca^{2+}. It is also caused by an increase in the intracellular second messenger inositol-1,4,5-triphosphate (IP3) [51]. These peptides cause activation of G-protein mediated mechanisms, stimulation of phospholipase A2, C and D, mobilization of $Ca^{2+}$from mitochondria and sarcoplasmic reticulum. It also activates the ryanodine receptor, modulation of various enzymes, such as Na^{+}-K^{+}-ATPase of rat brain, induction of the mitochondrial permeability transition and cell death by necrosis and apoptosis [52]. Mastoparan induce apoptosis that is initiated by the Ca^{2+}release from intracellular release from intracellular stores via PLC and IP3. It also causes disruption of plasma membrane integrity occurs secondarily [53]. The mastoparans affect intracellular free Ca^{2+}concentration in human astrocytoma cells [54] and inhibit NMDA receptor-mediated responses and block neurotransmission [55]. Functional activities of Ca^{2+}dependent K^{+} channels increase in basilar artery during chronic hypertension caused by wasp envenomation [56]. Mastoparan, an activator of GI and mast cells, selectively stimulates an PLD2, independently of GI, ADP-ribosylation factor-1 (ARF-1), protein kinase C and calcium in intact cells [57]. PLD is involved in the exocytosis of secretory granules of mast cells and neutrophils [58]. Mastoparans found in wasp venom is a potent stimulator of exocytosis from diverse mammalian cells. It causes secretion of histamine from mast cells, serotonin from platelets, catecholamines from chromaffin cells and prolactin from the anterior pituitary [59].

(v) Wasp-kinins

Wasps contain a series of different bradykinin related peptides in the venom from different species. Wasp kinins are polypeptides (9-18 amino acid residues) containing a bradykinin-like sequence at the C-terminal. Wasp contains two kinins-bradykinin (BK) and lysyl-bradykinin (table 1). These are produced in human plasma kallikreins and tissue kallikreins, respectively. These are important mediators of inflammatory responses, potent pain producers and increase vascular permeability and vasodilatation [59-61]. A nonapeptide bradykinin found within the wasp-kinin show potent pharmacological actions and long-lasting effects. Kinins, such as threonine-bradykinin (Thr6-BK) and megascoliakinin (Thr6-BK-Lys-Ala) and glycosylated wasp-kinins are neurotoxic. Wasp-kinins are experimentally involved in constriction and relaxation of muscles, activation of leukocytes followed by a release of cytokines, prostaglandins, leukotrienes, reactive oxygen species and the blockage of the cholinergic transmission in the insect central nervous system [61].

Bradykinin a non-peptide also found in body secretions such as urine, saliva and sweat. They are also found in several tissues such as heart, vasculature, blood, kidney, colon and liver [62]. Bradykinin is a potent endothelium-dependent vasodilator and mild diuretic, which may cause a lowering of the blood pressure. It also causes contraction of non-vascular smooth muscle in the bronchus and gut, increases vascular permeability and is also involved in the mechanism of pain. In addition to wasp kinins, the wasp venom contains a series of hydrophobic peptides, mastoparans and chemotactic peptides as major peptidergic components. The first major component in the venom is mastoparam. The peptides in the mastoparan family are tetradecapeptide amides, which cause degranulation of the mast cells to release histamine from the cells, and act on the adrenal chromaffin cells to release catecholamines and adenylic acids. Some mastoparans cause hemolysis and serotonin release from the platelets. The new cytotrophic peptides as the second major components are tridecapeptide amides possessing chemotactic activity for polymorphonuclear leukocytes and monocytes. Some of the peptides in this family also cause histamine release from the mast cells. Mastoparan takes a random coil structure in aqueous solution but changes its conformation to alpha-helix in methanol solution or in the presence of lysophosphatidyl choline [62] (table 1).

(vi) Other bioactive molecules

There are many other bioactive molecules in the wasp venom such as histamine, 5-HT, acetylcholine, tyramine, catecholamines, and various peptides. These are only a minor portion of the wasp venom. Peptides are major components mastoparans, wasp kinins and phospholipases [62]. Besides, these several other peptides include protonectin, mandaratoxins and chemotactic peptides also found which recruit macrophages and polymorphonuclear leukocytes near the site of stinging. Protonectins are the mast cell degranulating peptides responsible for histamine release. Fewer toxins from the honey bees and yellow jacket venom vitellogenins are designated as allergens API m 12 and vies v 6 respectively [63]. Their activities are very diverse and range from neurotoxic and inflammatory of antibacterial activity [64] (table 1).

Diagnosis of venom induced allergy

For diagnosis of bees, wasps, and other hymenopteran insect induced venom allergy skin and blood tests are performed. These tests reveal the presence of allergen-specific IgE antibodies in the serum [65]. Skin tests, mainly prick tests and intradermal tests are performed at least two weeks after the stinging episode. There remains a possibility a risk of a false negative result due to the refractory period. For evaluation of sensitivity medical history of patients is used to establish a diagnosis. Both blood and skin allergy tests can detect a patient's sensitivity to insect venoms.

Skin prick testing

Skin prick test (SPT) is usually done to identify venom induced allergies, mainly based on few systemic reactions [66]. A skin prick test is used to identify allergens responsible for triggering symptoms in the form of allergic diseases. In SPT skin is punctured or scratched by using purified allergen and detect immediate allergic reactions. Scratching in adults is usually done on the forearm. Skin prick testing is useful in the diagnosis of other allergies such as allergens found in bee and wasp venom. Skin prick testing is most often used to demonstrate atopy or an overactive immune response to environmental factors or to an allergen or immunogens. In skin prick tests reactions are assessed by the degree of redness and swelling and the size of the weal produced. A white, raised edge wheel is formed that surrounds the swollen, red central area of any skin reaction. Wheal size usually takes about 15-20 min to reach a maximum size and thereafter fades over a few hours. The weal size in mm of<4 shows a negative result, 5–10-mild sensitive, 10–15-moderately sensitive, and the largest wheal of 10–15 mm size shows high sensitivity to allergens (table 2).

RAST inhibition test

RAST or radioallergosorbent test is a radioimmunoassay that detects specific IgE or siege antibodies in response to allergen and for diagnosis of allergy. RAST (radioallergosorption test) test is performed to differentiate genuine dual sensitivity (being an indication to immunotherapy with vaccines made of both venoms) and cross-reactivity to hyaluronidase or CCD epitopes. The precision of results is highly difficult in such tests [67]. Obviously, diagnosis of allergy depends on the generation of component-specific IgE and monitoring the effects of venom immunotherapy in victim [68-70]. It was further modified to make more sensitive and named as FAST and ImmunoCAP [71, 72]. This test shows very high specificity as it binds to allergen-specific IgE, and extremely sensitive. IgE is the antibody associated with Type I allergic response. False-positive results may be obtained due to cross-reactivity of homologous proteins or by cross-reactive carbohydrate determinants (CCDs) [73]. ImmunoCAP and other RAST techniques take longer to perform and are less cost-effective. An allergic person remains positive as IgE persists for years after exposure. The RAST is scored on a scale from 0 to 6 (table 2).

Table 1: Important allergens found in hymenopteran venoms

Hymenopterans species	Allergens	Allergens name	Mol wt. (kD)
Honey bee			
Apis cerena	Api c 1	Phospholipase	16
Apis mellifera	Api m1 Api c1	Phospholipase A2	17
Apis cerena	Api c 2	Hyaluronidase	45
Apis cerena	Api c 4	Melittin	3
Apis dorsata	Api d 1	Phospholipase A2	17
Apis dorsata	Api d 4	Melittin	3
Apis florae	Api fl 4	Melittin	3
Apis mellifera	Api m2	Hyalurodinase	45
Apis mellifera	Api m3	Acid phosphatase	49
Apis mellifera	Api m4	Melittin	3
Apis mellifera	Api m5	DPP IV peptidases IV	100
Apis mellifera A cerana	Api m6	Protease inhibitor	8
Apis mellifera	Api m7	Protease	39
Apis mellifera	Api m8	Carboxylestrase	70
Apis mellifera	Api m9	Carboxypeptidase	60
Apis mellifera	Api m10	CRP/carpin	55
Apis mellifera	Api m 11 0101	MRJP8	65
Apis mellifera	Api m 11 0201	MRJP9	60
Apis mellifera	Api m12	Vitellogenin	200
Apis mellifera, A cerana	Recombinant MELT	Melittin Allergen Api m 3	2.8
Apis dorsata	PLA_2	phospholipase-2	39
Apium graveolens	Recombinant Major allergen Api g 1	Profilin	19
Wasps			
Vespula vulgaris	Ves v1 Ves m1, Ves s1	Phospholipase A1	35
V flavopilosa	Ves v2 0101 Ves m2	Hyalurodinase	45
V germanica	Ves v2 0201	Hyalurodinase	45
V maculifrons	Ves v3	DPP IV	100
V pensylvanica	Ves v5 Ves	Antigen 5	25
V squamosa	Ves v6	Vitellogenin	200
Vespa crabo	Vesp c1, Vesp m1	Phospholipase A1	34
V mangifica	Vesp ma2	Hyalurodinase	35
V Mandainia	Vesp c5, Vesp ma 5	Antigen 5	23
Hornets			
White-faced hornet *Dolichovespula maculate*	Dol m1	Phospholipase A1	34
White-faced hornet *Dolichovespula maculate*	Dol m2	Hyalurodinase	42
Yellow hornet *D arenaria*	Dol m5, Dol a 5	Antigen 5	23
American paper wasps			
Polistes annularis	Pol a1 Pol e 1		34
P exclamans	Pol a2		38
P fuscatus	Pol a4-		
P metricus	Pol a 5, Pole5		23
European paper wasps			
Polistes dominula	Pol d1 Pol g1	Phospholipase	34
Polistes dominula	Pol d4	Protease	33
P gallicus	Pol d5, Pol g5	Antigen 5	23
Fire ants			
Solenopsis Invicta	Soli 1	Phospholipase A1	35
S germinate	Soli 2, Solg2, Sol r2 Sol s 2		14
S richteri	Soli 3, Solg3, Sol r3 Sol s3	Antigen 5	26
S saevissima	Soli 4, Sol g4		12

Table 2: Categories of systemic allergic reactions to bee or wasp stings

Allergy level	Wheal size	Allergic reactions	Pathological changes/symptoms	References
Mild+	5–10	Erythema, pruritis urticaria, angioedema, rhinitis, nausea	Intense pain, mild to moderate local swelling with redness around the sting site, severe inflammation at the sting site.	[39]
Moderate++	10-15	Asthma, angioedema, abdominal pain	Redness and swelling extends beyond the sting site, persists for a couple of days. Take 5-10 d in improvement	[40]
Severe+++	15-20		Patient exhibit wheezing or gasping, dizziness, sudden drop in blood pressure, loss of consciousness, severe breathing or respiratory difficulties, sharp drop in blood pressure, fast pulse and dizziness	[65]

Immulite

IMMULITE test shows specificity to insect venom allergen and sensitivity [74]. It also displays the presence of IgE against both bee and wasp venom. This test is used to explore real cross-reactivity in allergic patients to insect venoms. Further, true cross-reactivity is displayed as react with carbohydrate epitopes (CCD–Carbohydrate Cross-reactive Determinants) of allergens. Positive IgE to both bee and wasp venom is often due to cross-reactivity to cross-reactive carbohydrate-determining reagents (CCD) [75]. A few specific tests

based on recombinant allergens (lacking CCD), are also developed for the diagnosis of allergy [75-77]. IMMULITE 2000 Allergy is a unique combination of chemiluminescence and liquid allergen technology. Both chemiluminescent enzyme immunoassay (CLEIA) and CAP test are allergen-specific IgE (sIgE) and assists in asthma, allergic rhinitis and atopic dermatitis. For detection of IgM antibodies in serum ELISA technique is used [78].

For skin testing and serological diagnosis IgE recombinant bee and wasp venom allergens are used [79]. Antigen 5 (~23 kDa) from *Polybia paulista* venom (Poly p 5) is a highly abundant and glycosylated allergenic protein that could be used for the development of component-resolved diagnosis (CRD). Soluble rPoly p 5 a recombinant allergen represents a potential candidate for molecular diagnosis of *Pichia pastoris* venom allergy [80]. The use of recombinant rapid m 1 and reveals v 5 with the LITE system, enhance the diagnostic sensitivity of Immulite testing system. This diagnostic utility of venom recombinants has improved allergy testing [81]. Level of recombinant allergens used assists in interpretation of the test results in routine clinical practice [82]. Asymptomatic allergy constitutes an additional significant issue. Elevated serum levels of sIgE quite frequently detect in individuals with no history of hypersensitivity. *In vitro* laboratory tests for sIgE antibodies together with the skin-prick test (SPT) are routinely used in the diagnosis of allergic disorders initiated through a type I hypersensitivity mechanism. However, the presence of sIgE is not 100% specific for the development of symptoms upon allergen exposure, but its presence can be viewed as a significant risk factor.

Basophil activation test (BAT)

The BAT is a flow cytometry-based assay where the expression of activation markers is measured on the surface of basophils following stimulation with the allergen. A positive basophil activation test can be seen as an *in vitro* surrogate of an acute allergic reaction *in vivo* Basophil activation test (BAT) test is applied for identification of primary sensitizing antigen in Hymenoptera venom allergy [83]. This test is based on the activation of patient-derived basophils by allergens. In this test, whole blood is stimulated with venoms or single allergens and the subsequent activation of basophil is measured by the detection of CD63 upregulation on the surface of basophil by flow cytometry technique [84]. BAT assists in diagnosis of venom allergy in mastocytosis patients with negative sIgE and low total IgE. Both side effects and allergen-specific tolerance are most important before VIT [85].

Component-resolved diagnosis

Component-resolved diagnosis (CRD) is the testing of specific IgE reactivity against several single allergens is evolving as a superior tool to support classical allergy testing. IgE antibodies directed against glycol structures of insect and plant proteins were shown to be of high affinity, but their clinical relevance seems to be low, meaning that for an unknown reason they are causing no clinical symptoms. Component-resolved diagnostics (CRD) is a diagnostic approach that utilizes purified native or recombinant allergens to detect the sIgE antibodies response against the individual allergenic molecule after allergen immunotherapy and to assess venom induced anaphylaxis.

Venom specific immunotherapy

Allergy to bee and wasp venom can lead to life-threatening systemic reactions. For the treatment of bee and wasp venom, envenomation venom Immunotherapy (VIT) is applied without any delay (table 3). It consists of subcutaneous injections of the increasing amount of purified bee and wasp venom extract [86]. Venom-specific immunotherapy is highly effective in preventing allergic reactions to insect stings and well-tolerated method for immunotherapy [87] (table 3). But the appropriate venom must be used to achieve clinical protection. Patients which show severe anaphylactic reactions, anti-immunoglobulin IgE monoclonal antibody omalizumab and VIT therapy is used [88] (table 3). Few anti-allergic drugs, antihistamines and corticosteroids are also provided to sting patients [89] only when they show sting derived-clinical manifestations [90]. Anaphylactic side effects are more common among patients with mastocytosis [91], in rare cases, a very severe reaction can arise [92]. Recurrent systemic anaphylactic side effects are rarely a sign of treatment failure. In patients with multiple positive results to venoms, molecular allergy diagnostics or CAP-inhibition may identify the cause venomous. Sometimes special clinical protection is required when the victim is attacked and stung by two different insect species [93].

Till date so many major toxins/allergens have been identified in many species of wasps and bees, which are of very high medical importance. There is need of molecular docking of important data on toxins for development of effective therapeutics through combination of transcriptomic, proteomic, peptidomic, glycomic and venomic approaches [94]. More often, the data available in various databases [95] on peptide toxins [96] can be used to develop new diagnostic and therapeutic approaches for treatment of poisonous animal stings and bites. Production of anti-venom serum against important toxins/allergens can be used in immunotherapy to encounter envenomation [97]. It is fact that for treatment of severe allergies or allergies may not be completely relieved by other treatments, hence, allergen immunotherapy can be applied. It involves a series of injections of purified allergen extracts, usually given over a period of a few years (table 3).

Table 3: Markers of venom immunotherapy

Types of reaction	Venom-specific IgE	Venom immunotherapy	References
Severe systemic cardiovascular effects, calcium influx disturbs	Positive	Yes	[84]
Moderate systemic light-headedness (angio-oedema, mild asthma or	Positive	Sometimes, but usually not	[87]
Mild systemic urticaria, angio-oedema	Positive	No	[93]

CONCLUSION

Stinging by insects is known to cause life-threatening allergic reactions and impair life quality. Insect venoms are harmful as they cause severe toxic and allergic immune effects which persists for longer duration. After infliction of venom victims show allergic response due to protein in the insect's saliva and insect venom. It causes an immediate allergic reaction and delayed reactions. Venom toxins generate strong T-cell responses in hypersensitivity patients and interact with IgE antibody molecules. Massive envenomation causes death in non-allergic individuals due to the toxic effects of the venom. Therefore, to enumerate the allergen-induced local and systemic effects and immune responses in victims is highly important. Therefore, the development of diagnostic methods able to predict the severity of the reaction to future stinging episodes is highly needful. Though so many conventional methods are available but their precision is not conclusive. However, recombinant antigens are to be used to improve both diagnostic and therapeutic methods. It will essentially require for substantial improvement of diagnosis and therapy of venom allergy.

Despite the effectiveness of conventional vespid venom immunotherapy, more standardized and safer allergy vaccines are required and recombinants hypo-allergic variants were found important clinical tools. Venom immunotherapy can restore normal immunity against venom allergens. It may also provide lifetime tolerance against venoms. Hence, it is highly essential and needful to monitor the development of tolerance in patients after the VIT therapy to avoid fatal anaphylactic reactions. Wasp toxins can be used as the most appropriate drug molecules against emerging and re-emerging viruses. But it will essentially need the establishment of biological activity, validation and clinical development of peptide toxins. For proper therapeutics training of health officials is highly required for proper diagnosis and management of allergic patients. Venom-specific immunotherapy is highly effective as it restores pathological effects and causes its reversal.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflicts of interest regarding the publication of this paper.

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