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WASP VENOM GENERATED TOXIC EFFECTS, ALLERGIC IMMUNE RESPONSES AND IMMUNOTHERAPY.

Krishna Kumar Prajapati and Ravi Kant Upadhyay*

Department of Zoology, D D U Gorakhpur University, Gorakhpur, 273009. U.P. India.

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***Corresponding Author**
Ravi Kant Upadhyay
Department of Zoology, D D U Gorakhpur University, Gorakhpur, 273009. U.P. India.

ABSTRACT

Present review article aims to explain wasp venom toxins, its action on gated channels with toxic and immune allergic responses generated after envenomation. Wasp venom contains mixture of various bioactive compounds of diverse nature and function. It contains consist of few peptides certain elements, acids, amines, peptides mainly pain producing peptides such as kinins and chemotactic peptides like mastoparans or crabrolin and enzyme proteins including many types of hydralases (proteases, hyluronidase, phosphatases, nucleotidases, phospholypases A) as well as allergens and neurotoxins. Present review article emphasize wasp venom induced symptoms, inhibitory effects on electro physiology of ion channels and various receptors. It also describes wide range of physiological effects on nerve transmission, ionic regulation, electrophysiological and serological changes. Wasp venom toxic substances to infiltrate the tissues and rupture the blood cells and generate IgE mediated hypersensitivity and toxicity to the tissues. This review also describes non-allosteric interaction of wasp venom toxins to receptor binding sites to synaptic vesicles, and voltage dependent antagonism, inhibition of nicotinic acetylcholine receptor, and selective blocking of calcium-permeable AMPA receptors. This review also suggests therapeutic and pharmacological uses of wasp venom templates for production bio-insecticides, antibiotics and analgesics. The natural substances purified from wasp venom can be used as important pharmacological tools or powerful probes for diagnostic techniques to explore complex biological processes of pharmaceutical importance. Various toxin structures from wasp venom can be used to make new drug design on suitable three-dimensional templates for creating small molecules/ or pharmaceuticals of great therapeutic importance.

KEYWORDS: Wasp venom toxins, peptides, proteins, channel inhibitors, allergens, immunotherapy.

INTRODUCTION

Animals inflict venom to maintain self-defense for predation and protection of territory. Stinging is highly adaptive evolutionary mechanism which is operated by using different defense organs such as fangs, nematocysts, spines, stings and pincers etc. Envenomation and poisoning by terrestrial animals mainly insects is a significant problem for human health. Usually wasps make lethal attack in large numbers and inject their venom toxins into the body of mammals mainly man and his pets.[1] The wasp venom is a toxic substance that causes severe inflammation, pain and allergic reactions after infliction in farmers, researchers, free dwellers, rural and urban people. Wasp venom toxins are unique in nature which act upon various ion channels and do its electrophysiological inhibition. Tarai region of eastern U.P. possess high bio-diversity of wasps which show more frequent interactions with man and his pets, thousands of wasp venoming cases are reported for medical aids every year. Wasp stinging impose multisystem changes and show wide range of biological effects such as intravascular hemolysis, rhabdomylysis, acute renal failure, cardiac involvement, hepatic dysfunction, thrombocytopenia and coagulopathy. Wasp venom toxins generate toxic effects with multiple organ dysfunction followed by anaphylactic reaction.[2] It causes significant serological changes[3], severely effect blood biochemical parameters and generate toxic effects in man.[4] Wasp venom also causes scanty micturation, generalization, swelling, and respiratory distress.[5] Wasps inject venom in the body of disturbing elements for making self-defense and fight against potential competitors and predators. The venomous stinger of Hymenoptera sting apparatus contain venom up to 140µg of venom from which 3µg is released in per wasp sting. In the bee stings, the stinger and the venom apparatus usually remain in the skin and continue to release venom afterward, while wasps can usually retracts their stinger after the stings.

Venom toxins are proteinaceous in nature and consist of few peptides mixed with certain elements and acids. Predatory animals use these toxins as firing chemicals in self-defense and to immobilize the prey. Toxins are unique natural products synthesized by venoming animals, which cause multiple symptoms in stung animals and impart inhibitory effects on electro physiology of ion channels. Besides this, some effects produced by toxins in man are of an acute nature, highly injurious and fatal. These toxic effects are readily discernible but very

few natural antibodies for these poisons are known. Chemicals have not been found effective against envenomation, which might successfully nullify the effect of venom. The natural substances can be used as important pharmacological tools or powerful probes or diagnostic techniques for the elucidation of complex biological processes of pharmaceutical importance. Currently, various toxin structures are used to drug design suitable three-dimensional templates for creating small molecules, which might mimick interesting pharmacological properties. It is a worldwide problem that toxin-bearing organisms constitute a potential public health problem with economic and social implications.

Wasp venom is a rich source of bioactive compounds that has been evolved to capture prey and make defense against predators and/or microorganisms. Hymenoptera stings occur very frequently, however, usually they are not as dangerous but these generate severe morbidities in organ system. In a healthy (non-allergic) person the reaction after the sting is limited to the occurrence of minor local symptoms. Moreover, people those who are highly allergic to the insect venom, showed severe local reaction and systemic symptoms including anaphylactic shock. Most patients with multiple wasp stings presented with toxic reactions and multiple organ dysfunctions rather than an anaphylactic reaction. AKI is the prominent clinical manifestation of the wasp sting with toxic reaction. Group attacks with repetitive stinging generate high creatinine levels, shocks, oliguria and anemia with additional risk of death. Wasp venom toxins are potent chemicals used as arsenal represents and a good source of new insecticidal compounds as they act selectively on their molecular targets. These toxins affect the nervous system of vertebrates and invertebrates and mainly act upon gated channels and demobilize them. Few invertebrates specific peptides neurotoxins that have been isolated from the venom of wasp which are used as good pest control agents and are also used as invaluable tools in neuropharmacology.[6] Several insecticidal compounds belonging to the class of peptides or polyamines like compounds have purified and characterized from the venom of arachnids and hymenoptera.

Parasitoid wasps are diverse and ecologically important insects that use venom to modify their host's metabolism for the benefits of the parasitoids off springs. The parasitoids wasp *Nasonia vitripennis* has approximately 100 venom proteins. Envenomation by *N. vitripennis* shows developmental arrest, selective apoptosis and alterations in the lipid metabolism in flesh fly hosts[7] while, mastoperans-induced programmed cell death (PCD) in the unicellular alga *Chlamydomonas reinhartii.*[8] Venom proteins from endo-parasitic wasps are

predominately involved in regulation of host physiology and immune responses alone or in combination with other factors of maternal origin such as polydnaviruses (PDVs) or virus-like particles presents in the venom itself or produced in the ovaries and ovarians fluids.[9] The venom, along with PDVs produced in the reproductive system of this wasp, are essential for successful parasitism as they protect the parasitoid from encapsulation by the host's immune cells[10], and interfere with the host's nutritional physiology.[11]

Chemical nature of venom toxins

Hymenopteran venom is a complex mixture of protein, polypeptides aromatic and aliphatic constituents in variable amount of enzymes like phospholipase α-2, hyaluronidase, acid phosphatase and α-D glucosidase which are strongly antigenic in nature. These are constituted by a complex mixture of chemically or pharmacologically bioactive agents such as phospholipases, hyaluronidase and mastoparans. Similar to other hymenopterans wasp venom consists of chemical complex mixtures of active amines (serotonin, histamine, tyramine, dopamine, noradrenaline and adrenaline), peptides (pain producing peptides such as kinins and chemotactic peptides like mastoparans or crabrolin) and proteins including many types of hydralases (proteases, hyluronidase, phosphatases, nucleotidases, phospholypases A) as well as allergens and neurotoxins.[12] These are complex cocktails, consisting of several hundreds of different components and lethal toxins. Venomous animals use stinging for hunting the prey. Wasp venom toxins possess a number of charged amino acids that have been shown too critical for their biological activity. Venom toxins are peptides, generally 3-6 kDa in size containing between 2 and 4-disulphide bonds, in a highly stable inhibitors cysteine knot(ICK) motif.[13] Wasp venom contains both toxins[14] and allergens[15] which induce severe anaphylaxis in animals and man soon after stinging.[16] Most of the major allergens have been identified in the species of medical importance through a combination of transcriptomic, proteomic, peptidomic and glycomic and venomic approaches.[17] These could act as broad spectrum antiviral drugs which are urgently needed to treat individuals infected with new and re-emerging viruses, or with therapies (Table 1).

Wasp venom induced symptoms

Hymenoptera (wasp and honey bees) stings are not generally serious in nature, but can cause severe systemic medical complications, such as allergic reactions.[18] Wasp venom stinging generates mild swelling, redness and pain that last from several minutes to several hours. Most patients with multiple wasp stings show toxic reactions and multiple organ dysfunctions

rather than an anaphylactic reaction. In contrast, few people show high allergy to insect venom, display severe local reactions and systemic symptoms, including anaphylactic shock.[19] Wasp stinging cause acute kidney injury and show toxicity induced clinical manifestation in man. Wasp venom displays high creatinine levels, shocks, oliguria, and anemia and even causes death.[2] Venom toxins of *Polybia paulista* showed genotoxic and mutagenic effects. Polyamine toxin from wasp is potent open-channel blockers of ionotropic glutamate (IGlu) receptors that show selective ligand binding.[20] The α- amino-3 hydroxy 5-methyl-4 isoazole propionic acid receptors (AMPARs) are glutamate gated cation channels mediating the majority of fast excitatory synaptic transmission in the central nerves system. The polyamine toxins derived from wasps act as voltages dependent channel blockers of Ca^{2+} permeable AMPARs.[21] Cholinergic signaling plays important role in regulating the growth and regeneration of axons in the nervous system. The α-7, nicotinic receptor (α-7) can drive synaptic development and plasticity.[22]

The wasp venom is highly toxic to small mammals in which it causes tissue irritation, swelling and inflammation and pain. It causes early and delayed hypersensitivity, inflammatory reaction, necrosis and toxic complications in mammals and even invertebrates animals.[23] Generally wasp envenomation occurs after little disturbance occurred in near vicinity of their hive. Wasps react and respond very fast to make an attack on predators and mammals. These inflict venom in to the body of enemy within no time by opening venom apparatus and charge upon heavily for making self defense. Few hymenopteran insects such as bees, bumblebees, wasps, hornets and ants bear such toxins which have attracted interest because they represent a source of neuroactive compounds that are useful tools in neuroscience and pharmacological investigations. No doubt wasp venom toxins are of very high pharmacological importance.

Patho-physiological effects of hymenoptera venom

Wasp venom toxins cause significant serological changes[3] and impose multi system changes and show wide range of activities such as intravascular hemolysis, rhabdomylysis. A sudden envenomation causes acute renal failure, cardiac involvement, hepatic dysfuntion and occasionally thrombocytopenia and coagulopathy. In experimental animals venom administration severally effects blood biochemical parameters and generates toxic effects.[24] The most common type of reaction to an insect sting is a local reaction to the bite of a mosquito (a small fly of the family Culicidae). The reaction reflects an allergic response to

proteins in the insect saliva leading in about ¾ of all to an immediate allergic reaction (wheal) and about ½ to a delayed reaction (papule). Hymenopteran insects induce IgE-mediated systemic allergic reactions which are of greater clinical significance. They show an immediate (anaphylactic) response that can have fetal consequences. Certain species of wasp belonging to family Vespidae example *Vespula vulgaris* and *Vespula germanica* cause anaphylaxis that is occasionally caused by others species of Vespidae such as *Dolicho vespula* species, hornet *Vespa crabro* and bees (mainly *bumblebees*).[25]

Besides the above, a variety of venoms and toxins, secreted by different animals, produce circulatory failure by different mechanisms. The important are direct cardiotoxicity, coronary vasospasm, hyperkalemia, pulmonary edema, and hypo-volumic shock with internal hemorrhages and intravascular clotting. Generally, in case of a severe bite, sodium, potassium ATPase pump gets shut off and edema rapidly spreads with extravasations of blood, epistaxis and petechie, abdominal pain, paralytic ileus, shock, albuminuria and prolonged clotting time. Major physiological symptoms, that appear after envenomation are local pain, edema, echymoses, necrosis and sloughing, restlessness, weak pulse, dyspnea; gastrointestinal, lymphatic, brain and mucous membrane hemorrhages, vomiting, abdominal pain and impaired liver function. In heavy and multiple repetitive envenomation loss of consciousness, failure of eye pupils and circulatory myonecrosis are most commonly observed. In addition, cardiovascular effects are also visualized in the form of pulmonary edema, acute metabolic acidosis, intracranial hypertension, hypothermia, and catecholamine increase in creatine kinase level and local lesion in skin with dark redness(Table 1).

The wasp venom is a complex mixture that contains diverse, more or less specific protein components.[26] Parasitoid wasps are among the most diverse group of insects on earth with many species causing major mortality in host populations. Parasitoids introduce a variety of factors into hosts to promote parasitism including symbiotic viruses, venom, teratocytes and wasps larvae[27] sensitization to hymenoptera venoms. IgE-level increase after the sting causes symptomatic sensitization.[3] Mast cells (MC) are effectors cells during severe systemic reaction (SR) to hymenoptera stings. Tryptase and Prostaglandin D2 metabolites (PGDs) are the markers of MC activation.[28] In children with IgE-mediated SR to hymenoptera stings elevation of baseline values of PGDs metabolites in blood is accompanied by decreased excretion of its urinary metabolites. Assessments of stable PGDs metabolites might serve as independent MC markers to identify allergic children. This is an

association between urinary PGD2 metabolites and severity of the SR to hymenoptera stings.[29] The endoparasitoid wasps are important natural enemies of the widely distributed aphid pests and are mainly used as biological control agents.

The venom of social wasp evolved to be used as defensive tools to protect the colonies against attacks of predators. Wasp venom comprises altogether up to 70% of the weight of freeze dried venoms.[30] Wasp peptides exists in a natural equilibrium between the form of oxidized with an intra-molecular disulphide bridge and reduce in which the thiol group of the cystiene residues do not form the disulphide bridge.[31] Some develop outside (ectoparasitoids) and other inside (endoparasitoids) the body of an insects or other arthropods host and, depending on the species, various stages of the host can be parasitized (eggs, eggs-larval, larval, pupal and adult parasitoids). In ectoparasitoid species venom proteins often induces paralysis and regulate host development, metabolism and immune responses[32] (Table 1).

Hymenoptera venom contains proteins, polypeptides and low molecular weight aromatic and aliphatic constituents in variable amounts. It also contains some important enzymes i.e., phospholipase A, hyaluronidase, acid phosphatase and D-glucosidase that are highly antigenic. The hylarunidase acts as a spreading factor that allows the toxic substances to infiltrate the tissues and rupture the blood cells. Phospholipase A shows no general hypersensitivity and toxicity to the tissues but indirectly inhibits action of thrombokinase, dehydrogenase and transaminase[33] and also inhibits oxidative phosphorylation. Wasp envenomation causes multi organ dysfunction after large venom load received by patients. However, envenomation in-group is fatal to humans as it causes severe inflammation, swelling, rhabdomyelosis, renal-insufficiency and severe pain. After few seconds of envenomation, toxins cause heavy RBC hemolysis and damage nerve cells[34] and biochemical functions of enzymes and proteins are also inhibited. Wasp toxins cause allergy by immune stimulation of the body. Acid phosphatase is a major allergen in honeybee venom and its availability as recombinant protein may facilitate the development of improved diagnostic tests and immunotherapies for the envenomated patients. Venom toxins generate strong T cell responses in hypersensitivity patients and interact with IgE antibody molecules. APImb can be used in diagnosis and therapy of bee venom toxicity.[35] APImb also signifies production of specific immunoglobulin of E type antibodies after envenomation.

Hymenopteran venom isolated from social species such as bees, bumblebees, wasps, hornets and ants show peculiar effects in animals and man after envenoming. Wasp venoms are complicated cocktails, consisting of several hundred different components. Venom toxins are the primary actors for toxicity in animal venoms, particularly for invertebrates venoms.[23] Toxins are distinct from enzymes, larger proteins and non-peptidic components like alkaloids and polyamines, and toxins are responsible for much of the biological activity and pharmacological interest around animal venoms and poisons. Hymenoptera venoms are constituted by a complex mixture of chemically or pharmacologically bioactive agents, Venom from the Apoidea and Vespoidea are primarily made of proteins, but formicid venoms are 95% alkaloids.[36] Wasp venom shows antimicrobial, insecticidal and hemolytic properties.[37] The crude venom of *Paulibia paulista* venom shows significant levels of hyaluronidase, phospholipase, and protolytic, hemolytic, and myotoxic reactions or activities.[38]

Wasp venoms consists of complex mixture of active amines (serotonin, histamine, tyrosine, dopamine, noradrenaline and adrenaline), peptides such as (pain producing peptides such as kinins and chemotactic peptides like mastoparans or crabroline) and protiens including many types of hyluronidases i.e., proteases, hyaluronidases, phospholipases A, nucleiotidases and phosphatases as well as allergens and neurotoxins. Wasp sting impose multiple changes and shows wide-range of biological effects such as intravascular hemolysis, rabdomylysis, acute renal failure, cardiac involvement, hepatic dysfunction, trombocytopenia and caugulopathy. The social wasp *Polibia paulista* suddenly sting and show serious allergic reactions that develop into anaphylactic shocks.[39] The social wasp is thus of great medical importance. Venoms can also contain substances that are able to inhibit and\or diminish the genotoxic or mutagenic action of other compounds that are capable of promoting damages in the genetic material.

Wasp sting also causes myocardial infarction that is a rare manifestation of acute coronary syndrome with other physiological changes.[40] Important pathophysiological alterations differ due to chemical composition of the venom. Few wasp venom toxins show vasoactive and thrombosis as well as allergic reaction in patients due to enzymes phospholipases A1, hyluronidases and acid phosphatases.[41] The vasoactive mediators histamine can activate platelets and potentiates. Heavy envenomation induces wasp venom allergy (WVA), severe life-threatening cardiopulmonary collapse and manifest initially as breathing difficulties,

bronchospasm, hypotension and arrhythmia.[42] Wasp venom immediate-type allergic reactions are represented by allergic rhinitis and conjunctivitis, bronchial asthma and atopic dermatitis. It also causes other reactions which are mediated by immunoglobulin IgE with variety of symptoms. Upon exposure wasp antigen IgE dependent allergy is induced from mast cells and basophils in the skin, the conjuctiva the mucosa of the oral cavity, nose and airway and the linking of the digestive tract(Table 1).

Inhibition of synaptic transmission

The digger wasp, *Philanthus triangulum*, which preys on honeybees, produces a paralysing venom possessing a wide variety of activities. In insects, venom showed a central as well as a peripheral effect; the latter effect consists of a presynaptic as well as a postsynaptic block of the skeletal neuromuscular transmission. The presynaptic block is probably caused by an inhibition of the re-uptake of the transmitter. The postsynaptic effect probably consists of a block of open ion channels. The venom contains at least four active toxins called alpha-, beta-, gamma- and delta-philanthotoxin (PTX). Alpha-PTX blocks transmission in the cockroach CNS. The other three toxins block neuromuscular transmission. delta-PTX being the most active toxin in blocking glutamate evoked postsynaptic depolarizations. In the junctional, as well as in the extrajunctional, muscle fibre membrane delta-PTX blocks ion channels in a use-dependent manner. Once the channel has been blocked, unblocking seems to be channels in a use-dependent manner. It may be semi-irreversible when agonist activation is low (spontaneous release of transmitter and/or leak of glutamate from the pipette.[43] Similarly, the venom of the solitary wasp *Philanthus triangulum* contains a cholinergic antagonist of the nicotinic receptor of the rectus abdominis muscle of the frog, *Xenopus laevis*. The venom of African *P. triangulum* contains two different cholinergic factors, a competitive and a non-competitive antagonist. The venom of the European *P. triangulum*may not contains a competitive antagonist of the nicotinic receptor of *X. laevis*, but only a very strong non-competitive antagonist. The possible non-synonymity of both groups of *P. triangulum* is discussed.[43] The venom of the wasp *Habrobracon hebetor* pre-synaptically blocks excitatory but not inhibitory neuromuscular transmission at locust skeletal muscle. Its mode of action on excitatory motor nerve terminals has been studied at the retractor unguis muscle of *Schistocerca* by means of ultrastructural stereology, paralysed and unparalysed preparations(Table 1).

Hemolytic effects

In addition, they showed weak hemolytic activity toward human erythrocytes. AMPs are widely distributed in different wasp venoms and might provide good templates for the development of novel peptide antibiotics.[44] The low-molecular-weight compounds of the venom of *Polybia paulista* include neuroactive peptides that can be used as pharmacological resources for anticonvulsant and anxiolytic drug research.[45] ICK venom toxins contain cysteine knot (ICK) motif are short peptides of generally 3-6 KDa in size containing between 2 and 4 disulphide bonds are strong hemolytic agents.[12] Wasp venom shows an accumulation of active principles which show diverse pharmacological effects. Many social insects have developed defensive system that prevent infection within their colonies. Wasp venom also shows anticonvulsants and anxialytic effects as well as cause alterations in the spontaneous behavior of the animals. Intra cerebroventricular injections of the wasp venom compounds induced dose-dependent anticonvulsants effect and potent anxiolytic activities.[45] Hornetin a highly basic protein (mol. wt. Of 32kDa) isolated form *Vespa flavitarsus* shows hemolytic activity in red blood cells and presynaptic neurotoxicity.[46] It also possesses catalytic activity to hydrolyze emulsified phosphatidylcholine but does not act upon sphingomyelin. Mellitin causes hyperalgesia in humans after envenomation.[47] Mellitin also interacts with RBC membranes and induces biochemical changes/disorders in lipid protein matrix both in hydrophobic core of lipid bilayer and at polar/non-polar interface of RBC membranes. Moreover, mellitin does make rigidization of lipid bilayer and alter reorganization of lipid assemblies and membrane protein rearrangements and consequently change the lipid protein interactions. Besides mellitin, citrate, present in venom inhibits phospholipase A2 activity[48] and capsaicin induces neuronal inflammation and causes hyperalgesia in mammals.[49] Histamine inhibits vaso-dilation in mast cells of lungs, liver and gastric mucosa with allergic hypersensitivity and inflammation in human body.

Neurotoxic effects

Wasp and bees venoms and their components are potential neuroprotectors or neuromodulators which can be used as alternatives for the control of the neurodegenerative diseases including Alzheimer's Disease, Parkinson's Diseases, Epilepsy, Multiple Sclerosis and Amyotropic Lateral Sclerosis.[50] Protonectins (ILGTILGLLKG-NH2), a peptides extracted from the venom of the wasp *Agenlaia pallipes pallipes* promotes mast cell degranulation activity. It also possesses catalytic activity to hydrolyse emulsified

phosphatidylcholin but does not act upon sphingomyelin. Wasp neurotoxins showed inhibitory and stimulatory actions on ion channels, receptors, and transporters involved in mammalian and insect neurotransmission.[51]

Allergic reactions and hypersensitivity:

On mammals, vespid venoms provoke prolonged pain, local oedema and erythema due to increased permeability of blood vessels in the skin. It shows generalized allergic reactions that may become lethal later on. Wasp and hornet venoms act kinetically on isolated smooth muscles and reduce blood pressure. They released endogenous histamines from granulocytes including mast cells and basophilic leucocytes and also released catecholamimes from adrenal chromaffin cells. Such toxins may be provokes cytolysis including haemolysis and chemotoxis to macrophases and polymorphonuclear leukocytes (Table 1).

Parasitoid wasps cause major mortality in the host population. These introduce a variety of factors into the hosts to promotes parasitism, including symbiotic viruses, venom teratocytes and wasp-larvae. Polydavirus-carrying wasps use viruses to globally suppress host immunity and prevent rejection of the developing parasites.[27] The normal reactions after sting includes such symptoms as: Mild-swelling, redness pain lasting etc from several minutes to several hours. In contrast, in people with allergy to insects venom, show severe local reactions and systemic symptoms, including anaphylactic shocks.[18] Although anaphylaxis due to rapid hypersensitivity is the primary concern with Hymenoptera venom that is mostly seen.[52] This hypersensitivity can be treated by using anti-IgE antibody (Ab).[53] Wasp venom sting induce local inflammation, pain and redness around the site of contact. The acute symptoms of an insects sting are treated asymptomatically.

Wasp venom generate severe allergy that is a potentially life-threatening condition with serious consequences of diagnostic error.[54] Its allergy generates an intradermal lesion which depends on individuals sensitization pattern.[55] The allergens most commonly causing IgE-mediated anaphylaxis are known phospholipase A2, hyluronidase and probably acid phosphate and a serine protease. Wasp venom, phospholipase A1, hyluronidase and antigen 5 are good allergens which cause hypersensitivity reactions. Acute kidney injuries (AKI) can develop after multiple wasp or bee stings. Wasp venom also causes acute tubular necrosis after secondary shock, pigment toxicity, interstitials nephritis or direct nephrotoxicity or acute Kidney injury (AKI) is developed after multiple wasp or bee stings venom. Wasp sting shows acute cortical necrosis (CAN) and dialysis[56], flexible bronchoscopy excluded lung

hemorrhage, hypocomplementemia in the case of anaphylaxis.[57] Bees and wasp venom differ in their composition, but the venom of *Vespa vulgaris* and *Vespa germanica* are very similar. Other pathological manifestation can arise on an immunological basis, occasionally in the form of a long-lasting disfiguring unusually severe local swelling accompanied by fever.[58]

Hymenoptera venom is a secretion of social poison glands of insects. It serves both as defensive substances against aggressors as well as weapon used to paralyze the victim during gaining food. Chemically, the venom is a mixture of biologically active substances of high-medium, and small molecular weight with a variety of physiological function.[59] Wasp venom generates an immediate allergic reaction (Wheel type) and delayed type reactions. But venom generates an immediate (anaphylactic) response that can have fatal consequences. It is commonly caused by the honey bee and certain species of wasp venom belong to the family Vespidae. The multiple stings, the toxin caused severe or even fatal illness. The serious conditions that are most often induced in this way are rhabdomyolysis, hemolysis, cerebral disturbances, hepatic and renal dysfunction. Anaphylactic due to hymenoptera stings is one of the most severe clinical outcomes of IgE-mediated hypersensitivity reactions. Few toxins from the honey bees and yellow jacket venom vitellogenin are designated as allergens Api m 12 and Ves v 6 respectivally.[60]

Wasp venom generates severe systemic reaction in childhood. These are related to mild eosinophilia, female sex and concomitant atopic diseases.[61] Venom from Hymenoptera display a wide range of functions and biological roles. It is an immune system disruptors, neurotoxics, cytolytic and highly painful with inflammation.[62] Venoms also contains substances that are able to inhibits/ diminish the genotoxic or mutagenic action of other compounds that are capable of promoting damages in the genetic materials.[63] Yellow jacket *Polibia poulista* (Pp-Hyal), wasp also generates severe venom toxin induced allergy.[54] It contains allergen hyaluronidase that shows high similarity (97%) with hyaluronidase from *Polistes annularis* venom (Q9U6V9).[64] Venom proteins contain signature sequences, including reprolysin like dipeptidyl peptidase 4, hyluronidase, and argine kinase or allergen protein. The venom extracts also contained novel proteins, encoded by venom genes conserved in Campopleginaeichneumonids and proteins with similarities to active molecules identified in others parasitoids species such as Calreticulin, reprolysin, superoxide dismutase and srepin [65] (Table 1).

Stinging by insects is known to cause life-threatening allergic reactions and impair life quality. *Polibias cutellaris* possesses hypoallergenic variants[66] which are useful to determine the patients individual risk profile. Persons who are frequently exposed to insects or who are predisposed to very severe anapylaxic are at higher risk. These patients show elevated basal level serum tryptase concentration.[67] Similarly, persons activated mast cells also remain at high risk of severe anaphylaxis.[68] Anaphylaxis reactions are also seen in more extreme cases with systemic symptoms involving the genitourinary tract or the nervous system. Cardiopulmonary system involvement may progress rapidly to life-threatening cardiopulmonary collapse and manifest initially as breathing difficulties, bronchospasm, hypotension and arrhythmia.[42] Myocardial infarction as result of wasp stings is a rare manifestation of acute coronary syndromes.[40]

The chemical composition of the venom made up by vasoactive and thrombogenic substances that able to create vasospasm and coronary thrombosis as well as bioactive allergenic venom protein such as phosphatases.[41] The vasoactive mediator, histamine can activate platelets and potentiates the aggregatory response of other agonists including adrenaline, 5-hydroxytryptamine, and thrombin. Histamine also induce pro-inflammatory cytokine production from endothetial cells.[69] The α-PMTX (α-pompilidotoxin) from wasp venom act on Na+ ion channels by slowing the channel inactivation without changing the peak current voltage relationship or the activation time course of the TTx-sensitive sodium ion currents. A-PMTX exhibited voltage dependent action with more rapid loss during stronger depolarization, similar to ATX-II. Furthermore the toxin showed voltage-dependent effects on the rate of recovery from inactivation and deactivating tail currents. It is possible that ATX-II or Tx Iα may act not only on the sodium ion channels sensitive to α-PMTX but also on some K+ chennels, and their blockage may cause marked prolongation of the action-potential in the axon[70] (Table 1).

Voltage-sensitive sodium ion channels are responsible for generating action potentials in most of excitable tissues. Membrane depolarization causes a voltage-dependent conformational changes that increases the permeability of Na+ ions, tissue-specific differences in the effects of sodium ion channel-specific neurotoxins indicates the existence of different subtypes.[71] A peptide toxin α-Pompilidotoxin (α-PMTX) found in the venom of solitary wasp *Anopolis samariensis*.[72] A-PMTX a 13-amino acids peptides, greatly facilitates both excitatory and inhibitory synaptic transmission in the lobster neuromuscular

synapse[72] as well as disrupts synchronous firing in rat cortical neurons[73], because the resting conductance of the postsynaptic membrane is not affected, α-PMTX appears to act mainly on the presynaptic membrane. The effects of α-PMTX were clearly different from those of known presynaptic toxins example-carybdotoxins or apanin, which affects Ca++ ions activated potassium ion channels.[72] Mammalian natural host defense peptides (mNHP) are short, usually cationic, peptides that have direct antimicrobial activity which is some instances activate cell-mediated antimicrobial activity immune responses.

Antimicrobial effects

Crude venom of *Vespa orientalis* exhibits strong antimicrobial activity against both gram-positive and gram-negative bacteria.[74] Wasp toxins show potent antimicrobial activity and can be used as effective antimicrobials to treat infectious diseases.[74] These also show insecticidal activity in small insect lepidopteran larvae. Wasp venom toxin shows low MICs values in micro broth kinetic system and in conventional broth microdilution.[75] AMPs isolated from *Vespa tropica venom* exert broad-spectrum antimicrobial activity against standard and isolated strains of bacteria[44] Similarly other wasp peptides, specifically mastoparan and some of its derivatives were found active against drug-resistant *Acinetobacter baumannii*[76], Analogues of mastoparan also act efficiently against resistant A. baumannii infections.[76] Protonectin (ILGTILGLLKGL-NH2), a peptide extracted from the venom of the wasp *Agelaia pallipes pallipes*, promotes mast cell degranulation activity, antibiosis against Gram-positive and -negative bacteria, and chemotaxis in polymorphonucleated leukocytes. [77]

The Polibia-MPI is cationic peptides from the venom of social wasp *Polibia paulista* that shows strong antifungal activity. It could potently inhibit the growth of the *Candida albicans* and *Candida glabrata*. The 50% inhibitory concentrations (IC 50%)of Polibia-MPI against cancer cells were much higher than the MICs against the tested *C. albicans* and *C. glabrata* cells, indicating the polibia-MPI had high selectivity between the fungal and mammalian cells.[78] Similarly, bee propolos and royal jelly present antimicrobial properties and the fecal pellets of termites inhibits the development of fungal pathogens.[79]

Anoplin (GLLKRIKTLL-NH2) an antimicrobial peptide (AMPs) is a promising candidate for battling multi-resistant bacteria.[80] These antimicrobial peptides (AMPs) from wasp venom showed broad spectrum antimicrobial activity and act through diverse mechanisms[81],

Mastoparan is an α-helical and amphipathic tetradecapeptide obtained from the venom of the wasp *Vespula lewisii*. This peptide exhibits a wide variety of biological effects, including antimicrobial activity, increased histamine release from mast cells, induction of a potent mitochondrial permeability transition and tumor cell cytotoxicity[82], anoplin-4 is a novel anoplin analogue with high antimicrobial activity and enzymatic stability, which may represent a potent agent for the treatment of infection[78] (R)-(-)-mellein in the headspace of parasitized cockroaches inhibited growth of entomopathogenic and opportunistic microbes (Serratia marcescens, Aspergillus sydowii, Metarhizium brunneum).[83]

Venoms of many animals are used as antimicrobials to treat infectious Wasp *(Vespa orientalis)* crude venom, shows diseases antibacterial effects against two gram-positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis)* and two gram-negative bacteria *(Escherichia coli* and *Klesiella pneumonia)* It could be considered a potential source for developing new antibacterial drugs.[74] Antimicrobial decapeptide anoplin shows strongly antifungal activity against plant pathogen *Leptosphaeria maculans* and shows protection *of Brassica napus* plants from disease.[84] The decapeptides anoplin (GLLKRIKTLL-NH2) is an especially interesting candidate because of its small size as well as its antimicrobial and nonhemolytic properties. Wasp venom shows antimicrobial effects against bacterial strains.[74] The venom exhibited broader inhibition zones 12.6, 22.7, 22.4 and 10.2 mm for *S. aureus, B.subtilis, E.coli* and *K. pneumonia*, respectively. The MIC values obtained were 64, 8, 64 and 128 µg/mL, respectively. European Hornet *Vespa crabro Linn.* showed lethal effects in small rodents.[13] Wasps can use their venom apparatus effectively when attacking foreign workers that appear in the immediate vicinity of their nest. The toxins released during stinging are potent enough to kill(Table 1).

Wasp venom toxins are also used as proves and extensively used as antibiotics agents against multidrug resistant microbes. Venom toxins can work as alternative medicine to replace synthetic antimicrobial agents resistant bacteria hornetin a highly basic protein (mol. Weight of 32 KDa) isolated from *Vespa flavitarrus* shows hemolytic activity in the red blood cells and presynaptic neurotoxicity.[85] It also possesses catalytic activity to hydrolyse emulsified phosphatidylcholine but does not acts upon sphingomyelin. Myolactone is a diffusible lipid secreted by the human pathogens *Mycobacterium ulcerans*, which induces the formation of open skin lesion refferd to as Burulliulcers.TheWiskott-Aldrich-Syndrome protein (WASP) family of actin-nucleating factors. By disruption of WASP auto inhibition, mycolactone-leads

to uncontrolled activation of ARP2/3-mediated assembly of actin in the cytoplasm.[86] Wasp venom including *Vespa crabro* possess antimicrobial[87] and chemotactic peptides (VCPs) such as mastoparans-VT1 and VCP-VT1. These inhibit bacterial growth and act as antibiotics.[88] These AMPs are widely distributed in different wasp venom and might provides templates for the development of novel peptides antibiotics.[44]

Mastoparans found in wasp venom is invertebrates host-defense peptides that penetrates lipid bilayers. Its analogs might interact with the lipid components of virus membrane and thereby reduce infectivity of enveloped viruses.[89] Wasp venom toxins are also used as probes and extensively used as antibiotics agents, multidrug resistant bacteria. Venom toxins can work as alternative medicine. Protonectin, a cationic peptide isolated from the venom of neotropical social wasp *Ageleia pallipes pallipes* exhibit of antibacterial activities by disruption of the integrity of the bacterial membrane, and its α-helical confirmation,[78] MitP, showed antimicrobial potential in vivo and can develop a potential antitumor drug with high selectivity. [90] Moreover, lipophilic anoplin analogs showed to be more active against *E. coli* and *Staphylococcus aureus* compared to native anoplin, while the EC-50 value of hemolysis was at least one order of magnitude lower than the MIC value. The incorporation of a lipophylic amino acids residue into anoplin enhanced the antimicrobial activities, while selectivity towards microbial membrane was retained.[91]

Vespa orientalis crude venom efficiently inhibited the gram positive and gram-negative bacterial strains, even at a very low concentration when compared to that of tetracycline. The crude venom showed to be more efficient again gram-positive bacteria. As the crude venom is comprised of different proteins and peptides, and found highly efficient against antibiotic-resistance pathogens.[74] *Polybia-MPI,* a cationic peptide isolated from the venom of social wasp *Polobia paulista* inhibits the growth of *Candida albicans.*[78] Polybia-MP1 (IDWKKLLDAAKQIL-NH2), a helical peptide extracted from the venom of a Brazilian wasp, has broad-spectrum antimicrobial activities without being hemolytic or cytotoxic[92] (Table 1).

Anti-cancer activity

Polibia-MPI a cationic peptide shows anticancer activity cancer cells.[78] It can be used as natural weapon to control progression of cancer due to their non-specific cytolytic activity as well as their rapid degradation and excretion when injected in blood. This a strong lytic peptides, if conjugated to poly(1-glutamic acid) PGA polymers, proteases, such as the

metalloproteinase-2 (MMP-2) or cathepsin B become more agent. Mitoparans are inactive compounds when they conjugated to the polymers and then become active again. Selective release of these cytolytic peptides inside tumor cells will provide exquisite spatiotemporal control.[93] Rhapontigenin has various biological activities including anticancer activities, suppresses breast cancer cell migration and invasion, which is involved in inhibiting the PI3K-dependent Rac1 signaling pathway.[94] Polybia-MP1 (IDWKKLLDAAKQIL-NH2), a helical peptide extracted from the venom of a Brazilian wasp displayed anticancer activity against cancer cell cultures.[92]

Venom Specific Immunotherapy

Hymenoptera venom anaphylaxis after bee and wasp sting is a common problem that affects about 1.2% to 3.5% of the general population. Venom specific immunotherapy (VIT) is an estimated mode of treatment for the immunoglobulin IgE-mediated Hymenoptera venom allergy. VIT may often be associated with immediate anaphylaxis which can lead to treatment withdrawal. Patients suffering from mild persistent asthma, severe anaphylactic reactions after yellow jacket hornet sting, (hypertension, dysphea, angioedema etc) for much patients immunotherapy is only treatment that can be reintroduced accompanied by the anti-immunoglobulin IgE monoclonal antibody omalizumab. For these patients combination of omalizumab and VIT is a valid option of therapy for these patients and could reduce asthma and food allergy symptoms.[16] VIT effectiveness can be estimated/verified by sting challenge (n=154) or the basis of BTC (Baseline serum tryptase concentration), age, gender, preventive use of anti-allergic drugs antihistamines and corticosteroids) right after a field sting.[68] *Vespa affinis* (thai banded tiger wasp) causes the most frequent incidence of the medically important Hymenoptera sting in South and Southeast Asia.

The venom components are attributable to the sting derived-clinical manifestations (Local reactions). Many short peptides from *V. affinis* venom have been identified that show allergy and serum IgE specific allergic reactions in patients.[95] Although anti- IgE antibody (Ab) therapy by wasp is find to be effective in patients with bronchial asthma it potentials prevent wasp venom anaphylaxis in patients. Anti-IgE-Ab treatment is an effective preventive measure against wasp venom induced anaphylaxis.[96] Venom components attributable to the sting derived- clinical manifestations (local reaction, IgE-mediated anaphylaxis or Systemic envenomation) are lacking.[95] Most patients with systemic anaphylactic reaction to bee and wasp stings need specific immunotherapy. Redness and swelling arise at the injection site in

almost all cases, usually in the escalation phase. It is not rare for patient to have a single episode of anaphylactic side effects, but such episode is usually mild and responsive to treatment. Anaphylactic side effects are more common among patients with mastocytosis [97], in rare cases, very severe reaction can arise.[98] Recurrent systemic anaphylactic side effects are rare a sign of treatment failure.

Mode of action

Physiologically animal toxins are highly active as they block various channels and breach the normal barrier for free movement of molecules across cell membrane. Venom toxins specifically act upon neurons, nicotinic acetylcholine receptors and neuromuscular junctions. A phospholipase toxin severely acts upon motor nerve terminals and muscle cells. It damages skeletal muscles and inhibits cell regeneration. Some toxins such as melletin are enzymatic in nature and hydrolyze membrane phospholipids and form channels through which small molecules may pass. Few toxins cause enormous hemolysis of RBCs and damage nerve cells. Some protease inhibitors occur in snake venom bind which to protease enzymes and prevent their action. The protease inhibitors also inhibit fibrin activity in arthritic joints and induce chronic arthritis. Various venom toxins isolated from snake venom induce immunomodulatory, cardio-respiratory, analgesic and hemopoeitic activity. Venoms also cause acute and chronic inflammatory responses in laboratory animals. Histamine inhibits vaso-dilation in mast cells of lungs, liver and gastric mucosa with allergic hypersensivity and inflammation. More specifically citrate present in arthropod venom inhibits phospholipase A2 activity [48] (Table 1).

Few seconds after envenomation, toxins cause sudden inflammation in body cells with a severe pain and do massive inhibition of axonal transmission in neurons. The activity of ATP driven Na^+-K^+ ATPase pump, which plays a key role in maintaining cell volume and intra cellular 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 helps the neurons to maintain 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.

Toxins also change the orientation and affinity of ion binding sites and change ion permeability mediated by the nicotinic Ach receptors. Snake neurotoxins such as α-bungarotoxin and cobra toxin block neuromuscular transmission; affect sodium and calcium exchanges and block ion channels forming a tight ring.[99] Different animal groups have different channel inhibitors. Some toxins are inhibitors of metabolic enzymes, and have hydrophobic pockets in their secondary structure by which they 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. Basically, site-specific mutations and rearrangements in the active site region in response to gradual environmental changes result in structural and functional diversification of toxins. Therefore, different biologically active toxins evolve within a single species. The mode of action of animal toxins and their physiological consequences vary greatly according to the structural variability in the active site region (Table 1).

Pharmacological effects of venom toxins

The polyamine toxins derived from wasps are used and voltage dependent channels blockers of calcium ions permeable AMPARs.[21] These are potent open-channels blockers of ionotropic glutamate (iGlu) receptors. The AMPA and NMDA receptors identify highly potent and in some cases very selective ligands.[20] The α-amino 3 hydroxy-5 Methyl-4-isoazole propionic acid receptors (AMPARs) are glutamate-gated cation channels mediated the majority of fast excitatory synaptic transmission in the central nervous system. More specifically, delta-Philanthotoxin, was found a potent a semi-irreversible blocker of ion-channels [43], Pompilidotoxins (PMTXs, alpha and beta) are small peptides consisting of 13 amino acids purified from the venom of the solitary wasps *Anoplius samariensis* (alpha-PMTX) and *Batozonellus maculifrons* (beta-PMTX) [100], These toxins showed voltage-gated sodium channel isoform-specific effects and are known to facilitate synaptic transmission in the lobster neuromuscular junction. These toxins instantly slowly did sodium channel inactivation. PMTXs mainly beta-PMTX, alpha-PMTX and four synthetic analogs showed sodium current inactivation in seven mammalian voltage-gated sodium channel (VGSC) isoforms and one insect VGSC (DmNa(v)1). Binding shows complex and intriguing behavior of VGSCs in response to PMTXs.).[100] Similarly, Polybia-MPII (INWLKLGKMVIDAL-NH2), a mastoparan isolated from the crude venom of the swarming wasp *Polybia paulista*, binds to motor end plate that is identified by the positive labelling of acetylcholine receptors by TRITC-alpha-bungarotoxin co-labelled with anti-synaptophysin

antibody. No doubt mastoparan Polybia-MPII is a minor neurotoxin that shows neurotoxic activity and is unlikely to be of clinical significance.[101] Similarly, wasp *Ampulex compressa* injects venom directly into the prothoracic ganglion of its cockroach host to induce a transient paralysis of the front legs. More specifically, Dabsylation of venom components produces transient block of synaptic transmission at the cercal-giant synapse and block efferent motor output generates in the prothoracic ganglion, it mimics effects produced by injection of whole venom. Contrary to this whole venom evokes picrotoxin-sensitive chloride currents in cockroach central neurons, consistent with a GABA allergic action.[102] Tuning wasp toxin is potent voltage-dependent blockers.[103] Ampulex utilizes GABAergic chloride channel activation as a strategy for central synaptic block to induce transient and focal leg paralysis in its host.[103]

Voltage dependent channels interactions

Polyamine toxins from wasps are potent open-channels blocker of ionotropic glutamate (iGlu) receptors. The AMPA and NMDA receptors identify highly potent and in some cases very selective ligands.[20] The alpha-amino 3-hydroxy-5-methyl-4- isoazol propionic acid receptors (AMPARs) are glutamate-gated cation channels mediating the majority of fast excitatory synaptic transmission in the central nervous system. The polyamine toxins derived from wasps are used and voltage dependent channels blockers of Ca2+ permeable AMPARs.[21] Cholinergic signaling plays an important role in regulating the growth and generation of axons in the nervous system. The alpha-7 nicotinic receptors can drive synaptic development and plasticity.[22] Wasp venom proteinacious and non-proteinaceous components can be used as agrichemicals as well as pharmaceuticals components to improves pest management or health related disorders[104] (Table 1).

Voltage-dependent antagonism

Solitary wasps, solitary bees, social wasps and ants showed effects on nicotinic acetylcholine receptors (nAChR) and ionotropic glutamate receptors (IGRs) of both the N-methyl-d-aspartate (NMDAR) and non-NMDAR type.[105] Solitary wasp venoms caused significant voltage-dependent antagonism of nAChR responses to 10 micro MACh and NMDAR responses to 100 micro MNMDA (+10 microM glycine) when co-applied at 1 microg/ml with the agonists.[146] At positive holding potentials (V(H)) potentiation of these receptors was observed with some venoms.[105] Solitary bee venoms only affected nAChR by causing either voltage-independent antagonism or potentiation of their responses to 10 microM ACh.

Of four social wasp venoms, one acted on nAChR by potentiating responses to 10 ACh, while another generated an ACh-like response when applied alone.[105] More often, presence of nAChR agonists and antagonists and NMDAR antagonists in Hymenopteran venoms showed presence of multiple nAChR venom components.[105] When synthetic analogues of delta-philanthotoxin (PhTX-433) and the polyamine spermine applied on the excitatory postsynaptic current (EPSC) of glutamatergic synapses and single channel currents gated by quisqualate-sensitive glutamate receptors (QUIS-R), generate both intracellularly and extracellularly effects on locust leg muscle. When applied extracellularly all 3 compounds reversibly antagonised the EPSC and the single channel currents. Moreover, toxins may bind to the closed and open channel conformations of QUIS-R at a site near the intracellular opening of the channel gated by this receptor [106] and antagonism was voltage independent, but agonist dependent. PTX-4.3.3, the synthetic product of the wasp toxin, delta-philanthotoxin, blocks the open, glutamate gated, ion channels of the locust muscle. This block is accompanied by the appearance of double exponential distribution of the closed times frequency, and a decrease of the inverse relationship between the open and closed states. 3. The analogues with the shortened polyamine chain, PTX-4.3 and PTX-3.3, are less potent, trifluoromethyl-PTX-4.3.3 is a more potent analogue. A long-term inhibition of PTX-4.3.3 applied at low concentrations also indicates that beside an open ion-channel block, also other inhibitory processes are involved [107] (Table 1).

Na (+) channel inhibitors

Wasp toxin pompilidotoxin (PMTX) PMTX inhibited inactivation of sodium currents and toxin binds at distinct places on the lobster axon and on the rat *hippocampal* cells. It is due to arrangement of polar and non-polar amino acids of the two toxins and found that similar sequence of PMTX exist in a discrete position of three-dimensional structure of ATXII. The sequence may be responsible for the binding site in the neuronal Na(+) channel molecule because PMTX is insensitive to cardiac Na(+) channel and come from other regions than the overlapped sequence. PMTX has diverse actions in the central neurons and is useful to classify Na(+) channel subtypes.[108] Pompilidotoxins (PMTXs), derived from the venom of solitary wasp has been known to facilitate synaptic transmission in the lobster neuromuscular junction.[109] PMTXs, which are small peptides with 13 amino acids, are used as potential tool for exploring a new functional moiety of Na+ channels.[109] Alpha-PMTX, a novel peptide toxin derived from wasp venom acts on neuromuscular synapse in the walking leg of the lobster. Alpha-PMTX is known to induce repetitive action potentials in the presynaptic axon

due to sodium channel inactivation. Effects of a wasp toxin Beta-pompilidotoxin (beta-PMTX) were also made on on rat *hippocampal* CA1 interneurons by the current-clamp technique. The firing patterns of pyramidal neurons and pyramidale interneurons were not affected by beta-PMTX, but in oriens and radiatum interneurons, beta-PMTX converted the action potentials to prolonged depolarizing potentials by slowing the inactivation of Na(+)channels. Moreover, beta-PMTX modulates Na(+) currents in CA1 interneurons differently in various CA1 neurons and the toxin is useful to classify Na(+) channel subtypes.[110] Moreover, 29 analogs of alpha-PMTX are prepared by substituting one or two amino acids and compared threshold concentrations. These mutant toxins are used for inducing repetitive action potentials. In 13 amino acid residues of alpha-PMTX, Arg-1, Lys-3 and Lys-12 regulate the toxic activity because substitution of these basic amino acid residues with other amino acid residues greatly changed the potency. PMTXs structure-activity relationships will assist in clarifying the molecular mechanism of sodium channel inactivation [72] (Table 1).

The parasitoid solitary wasp *Ampulex compressa* uses the cockroach *Periplaneta americana* as a food supply for its larvae. Wasp injects a venom cocktail into the brain of the cockroach to quickly paralyze and impose unusual effects on the behavior of its prey [111], The venom, reconstituted into the bilayer, showed ion channel activity, forming a fast-fluctuating channel with a small conductance of 20+/-0.1pS, with no voltage sensitivity. These channels were not observed when the venom was digested with proteases before application to the bilayer, but were not affected by exposure to protease after their incorporation into the bilayer, indicating that the active venom component is a peptide. The channels were found to be cation selective with similar selectivity for the mono valent cations K(+), Li(+) and Na(+), but showed high selectivity against anions (Cl(-)) and divalent cations (Ca(2+) and Mg(2+)[111] (Table 1).

Effect on Ca2+-conducting channels

Sarcoplasmic reticulum (SR) contains a Ca2+-conducting channel that is believed to play a central role in excitation-contraction coupling by releasing the Ca2+ necessary for muscle contraction.[159] The effects of calmodulin on single cardiac and skeletal muscle SR Ca2+-release channels.[112] Venom of the digger wasp *Philanthus triangulum F*. reduces the temperature- and voltage-sensitivity of the acetylcholine-receptor-activated ion channels, at the motor end-plate, and shortens the decay time of the miniature end-plate currents, analogous to a block found in toxin. Simlarly, delta-PTX, acts on insect glutamate-

activated channels and shows effect similar to that of the total venom on the decay phase of miniature end-plate currents.[113] The venom of the wasp *Habrobracon hebetor* pre-synaptically blocks excitatory but not inhibitory neuromuscular transmission at locust skeletal muscle. Its mode of action on motor nerve terminals retractor unguis muscle of *Schistocerca* is excitatory when it may remain either in resting or stimulated for 7 min at 20 Hz. Paralysis does not cause structural damage to the nerve terminals but prevents the depletion of vesicles occurring upon nerve stimulation in the controls. Prolonged paralysis leads to an increase in the number and the size of vesicles after 2 days. Stimulation causes swelling of mitochondria both in controls and in paralysed preparations, resulting from a rise of intraterminal [Ca2+] as is indicated by the absence of the swelling if extracellular Ca2+ is replaced by Mg2+. In addition, stimulation leads to a reduction of vesicle size, an increase in the area of axolemma and in the number of cisternae and of profiles of the smooth endoplasmic reticulum in controls but not in paralysed preparations. However, neither in controls nor in paralysed preparations is the total amount of membrane per terminal cross-section affected by stimulation. Under paralysis, vesicles tend to stick to the presynaptic membrane.[114] *Habrobracon* venom does not block the depolarizing-dependent Ca2+-influx into the nerve terminal and that it is unlikely to interfere with some transmitter-related process. Rather, the venom seems to block vesicle exocytosis itself. It clears involvement of insect neuromuscular synapses exocytosis is the mechanism whereby transmitter quanta are released[114] (Table 1). The venom toxins of many wasps bind to ion-voltage gated channels or receptors and affect their physiological functions by blocking them. Wasp toxins are potent voltage-dependent blockers[73] and glutamate receptors inhibitors which work as potential insecticides.[115] Wasp polyamines toxins act as inhibitors of the ionotropic glutamate receptors.[20] Philanthotoxins-1 causes pre and post-synaptic inhibition of the locust neuromuscular transmission.[107] Philanthotoxin-2 effects on the glutamate gated ion channels of the locust fibers membranes.[107] Wasp and bees venom impose severe systemic reactions in children[116] that varies differential gene expression profile in the venom gland or sac.[117] Wasp toxins generates resurgent like currents in mouse vas deferens myocytes which are mediated by NaV1.6 voltage-gated sodium channels.[118] Few of these toxins such as mastoparans shows dual effects as they effects intracellular free Ca++ concentration in human astrocytoma cells[119] and inhibit NMDA receptor-mediated responses and block neurotransmission.[120] Functional activities of Ca2+ dependent K+ channels are increased in basilar artery during chronic hypertension caused by wasp envenomation.[121] Philanthotoxins bind with nicotinic acetylcholine receptors[122], while β-pompilidotoxins generate resurgent

current in NaV1.6-null purkinje neurons by slowing sodium channels inactivation.[123] Symptoms depend on mode of interaction between toxins and receptors.[122] UV light speeds up restoration of the ionic channel activity and the synaptic transmission and thereby contributes to a more rapid awakening.[124] there are clear evidence that defencins have been transformed into neurotoxin during long evolution and adopted toxic functions.[125] Contrary to this, philanthotoxin analogs act upon N-methyl-D-Aspartate and nicotinic acetylcholine receptors.[126] Wasp toxins peptides mastoparans effects on calcium influx causes phosphoinositide breakdown and arachidonic acid release in rat pheochromocytoma PC12 cells.[127] Toxin peptides enhance Ca++ release from skeletal muscle sarcoplasmic reticulum[128], and affect intracellular Ca++ pools polyamines operate through channels gating mechanism and strong inward rectifier K channel Kir2.1.[129]

K^+ channel inhibitors

Rectifying K channels are essential for maintaining resting membrane potential and regulating excitability in many cell types.[129] Process of rectification initiates by strong inward rectifiers such as Kir2.1 to voltage-dependent binding of intracellular polyamines or Mg to the pore (direct open channel block), which prevent after voltage dependent binding of toxins outward passage of K ions is increased.[129] Thought, polyamine block is not consistent with direct open channel, but these interact to ion channel quickly and make closure.[129] Such responses also effect intracellular free calcium concentration ([Ca2+]i) and whole-cell calcium currents.[121] Philanthotoxin inhibits Ca2+ currents in rat hippocampal CA1 neurons.[130] PhTX-4.3.3 inhibits the N-methyl-D-aspartate (NMDA) transmission in rat hippocampus.[130] Philanthotoxins inhibit the calcium influx via voltage dependent as well as NMDA mediated calcium channels and thus reduce excitability in the hippocampus.[130] AMPAR block by polyamine toxins is modulated by auxillairy subunits from the class of transmembrane AMPAR regulatory proteins (TARPs).[21] Like the natural toxin, synthetic delta-philanthotoxin, designated as PTX-4.3.3 acts as a reversible post-synaptic open ion-channel blocker of the glutamatergic neuromuscular system of the locust. It inhibits the high-affinity re-uptake of glutamate in the nerve endings and glial cells. These toxins act as weak receptor antagonists. PTX-analogues, trifluoromethyl-PTX-4.3.3,is a potent postsynaptic blocker. The presynaptic inhibition of the glutamate re-uptake, (dehydroxy-PTX-4.3.3) exhibited this capacity.[130]

β-Pompilidotoxin, a voltage-gated Na(+) channel activator, evoked sustained resurgent-like currents in Na(V)1.6(+/+) which are generated as a result of Na(V)1.6 channel activity.[116] Venom peptide transcript shared sequence similarity with trypsin inhibitors and dendrotoxin-like venom peptides known to be K(+) channel blockers, causes paralysis. phospholipase A2 and hyaluronidase, are main components of wasp venoms. Several transcripts encoding enzymes, including three metallopeptidases and a decarboxylase likely involved in the processing and activation of venomous proteins.[131] Wasp venom toxins cause transient increase in insulin secretion Ca2++ concentration due to removal of extracellular Ca2+, by L-type voltage-dependent Ca2+ channel blockers.[132] Philanthotoxin-433 (PhTX-433) is a non-competitive channel blocker found in the venom of the wasp *Philanthus.* Its photolabile derivative (PhTX-433) interacts with nAChRs.[122] It may work as putative model of the binding of PhTx and related polyamine toxins to nAChRs in vitro[122] (Table 1).

Inhibition of glutamate and GABA uptake

AvTx 7 potentiates glutamate release in the presence of K(+) channel blockers tetraethyl-ammonium and 4-aminopyridine, indicating that the toxin may act through these drugs-sensible K(+) channels. Denatured crude extract of *Agelaia vicina* wasp venom inhibits glutamate and GABA uptake in rat cerebral cortex synaptosomes.[133] Effects of beta-pompilidotoxin (beta-PMTX), a neurotoxin derived from wasp venom, on synaptic transmission in the mammalian central nervous system (CNS). Beta-PMTX facilitates excitatory synaptic transmission by a presynaptic mechanism and that it causes over excitation followed by block of the activity of some population of interneurons which regulate the activity of GABA(A) receptors.[134] Bradykinins were identified in three solitary wasp venoms mainly in scoliid wasp *Megacampsomeris prismatica* led to the identification of bradykinin and threonine(6)-bradykinin as the major peptide components. The survey of a number of extracts from solitary wasp venom by MALDI-TOF MS revealed that the venoms of two other scoliid wasps, *Campsomeriella annulata annulata* and *Carinoscolia melanosoma fascinata*, also contained Thr(6)-BK as one of the major components. Bradykinins play a major role in their paralyzing action for prey capture because these have been shown to block the synaptic transmission of the nicotinic acetylcholine receptor in the insect central nervous system.[135]

Several wasp venoms contain philanthotoxins (PhTXs), natural polyamine amides, which act as noncompetitive inhibitors (NCIs) on the nicotinic acetylcholine receptor

(nAChR). Effects of varying the structure of PhTXs and poly(methylene tetramine)s on the binding affinity have been investigated. The stoichiometry of PhTX binding was determined to be two PhTX molecules per receptor monomer. PhTXs appeared to bind to a single class of non-allosterically interacting binding sites and bound PhTX was found to be completely displaced by well-characterized luminal NCIs. To elucidate the site of PhTX binding, a photolabile, radioactive PhTX derivative was photocross-linked to the nAChR in its closed channel conformation resulting in labeling yields for the two alpha and the beta, gamma and delta subunits of 10.4, 11.1, 4.0 and 7.4%, respectively. PhTXs and poly(methylene tetramine)s enter the receptor's ionic channel from the extracellular side. The hydrophobic head groups most likely bind to the high-affinity NCI site, while the positively charged polyamine chains presumably interact with the negatively charged selectivity filter.[136]

Selective blocking of calcium-permeable AMPA receptors

Using the polyamine toxin philanthotoxin, which selectively blocks calcium-permeable AMPA receptor synaptic transmission onto single *hippocampal* interneurons occurs by afferent-specific activation of philanthotoxin-sensitive and -insensitive AMPA receptors. Calcium-permeable AMPA receptors are found exclusively at synapses from mossy fibers. [137], Inward rectifying K channels are essential for maintaining resting membrane potential and regulating excitability in many cell types. Moreover, interactions between polyamines and the polyamine toxins philanthotoxin and argiotoxin on inward rectification in Kir2.1 shows high affinity polyamine block is not consistent with direct open channel block, but instead involves polyamines binding to another region of the channel (intrinsic gate) to form a blocking complex that occludes the pore. This interaction defines a novel mechanism of ion channel closure.[129] The wasp venom philanthotoxin-4.3.3 (PhTX-4.3.3) is an antagonist of glutamate transmission in the insect as well as in the mammalian brain. It was recently shown that PhTX-4.3.3 inhibits the N-methyl-D-aspartate (NMDA) transmission in rat *hippocampus*. In this study we show that dideaza-philanthotoxin-12 (dideaza-PhTX-12), an analogue of PhTX-4.3.3, is a potent antagonist of voltage-dependent Ca2+ currents in rat *hippocampal* CA1 neurons. At a concentration of 10 micro M it reduces the Ca2+ current to 40%. Two voltage-dependent potassium currents, the A current and the delayed rectifier, were hardly affected by dideaza-PhTX-12, indicating selectivity of the drug for Ca2+ currents. As a consequence the philanthotoxins will inhibit the calcium influx via voltage dependent as well as NMDA mediated calcium channels and thus reduce excitability in the *hippocampus*[130] (Table 1).

Wasp toxins are reversible postsynaptic open ion-channel blocker of the glutamatergic neuromuscular system. Like the natural toxin, synthetic delta-philanthotoxin, now called PTX-4.3.3 acts as a reversible postsynaptic open ion-channel blocker of the glutamatergic neuromuscular system of the locust. 2. It also inhibits the high-affinity re-uptake of glutamate in the nerve endings and microglial cells. Possibly these two toxins act as weak receptor antagonists. The presynaptic inhibition of the glutamate re-uptake, seemed to be a very specific property of PTX-4.3.3. Only one of the tested analogues (dehydroxy-PTX-4.3.3) exhibited this capacity.[138]

Inhibition of nicotinic acetylcholine receptor

PhTX interacts with two receptors and shows at least two binding sites: an external polyamine binding site and a channel binding site [139], Philanthotoxin (PhTX) that contains four moieties tyrosine, butyrate, spermine and the terminal amino group is potent channel blocker. Although, the potencies of the PhTX analogs on both receptors were higher with increasing lipophilicity and the polyamine chain length. It shows considerable divergence between the two receptors' channels in the structural activity requirements for blockade by PhTX analogs.[139] The venom of the solitary scoliid wasp *Colpa interrupta (F.)* shows a kinin-activity, when tested on a cascade of mammalian smooth muscle preparations, and, in addition, a contraction of the rat colon. The venom also irreversibly blocks the nicotinic synaptic transmission from the cercal nerve to a giant interneuron in the sixth abdominal ganglion of the cockroach, *Periplaneta americana.* Synthetic Thr-bradykinin causes irreversible presynaptic activation-induced block of transmission in the insect CNS [140] (Table 1).

Mastoparans

Mastoparan also causes closure of ATP-sensitive and potassium channels [K(ATP) channels] in cell-attached and excised membrane patches.[141] Mastoparan induces an increase in cytosolic calcium ion concentration and subsequent activation of protein kinases in tobacco suspension culture cells.[142] Impaired insulin secretion by diphenyleneiodium associated with perturbation of cytosolic Ca2+ dynamics in pancreatic beta-cells.[132] Mastoparan-stimulated prolactin secretion in rat pituitary gh3 cells involves activation of Gq/11 protiens.[133] Mastoparan increases the intracellular free calcium concentration in two insulin-secreting cell lines by inhibition of ATP- sensitive potassium chennels.[141]

Crude extract of *Agelaia vicina* wasp venom inhibits glutamate and GABA uptake in rat cerebral cortex synaptosomes [133], AvTx 7 inhibits glutamate uptake in a dose-dependent and uncompetitive manner but it stimulate the glutamate release in the presence of calcium and sodium channel blockers, which shows that AvTx action is not mediated through these channels. AvTx 7 potentiates glutamate release in the presence of K(+) channel blockers such as tetraethyl ammonium and 4-aminopyridine. It shows that indicating that the toxin may act through these drugs-sensible K(+) channels. AvTx 7 act upon K(+) channels involvement in the release of glutamate.[133] Mastoparans has been reported to induced a wide variety of cellular actions by activating GTP-binding proteins (G proteins) in various cells. Mastoparan is able to stimulate the secretion of PRL from rat anterior pituitary tumors GH3 cells in dose and time dependent manners. Mastoparans had no effects on the accumulation of intracellular cAMP; however, it induced a raspid incresese in the intracellular Ca2++ concentration in GH3 cells. Extracellular Ca2++ requires mastoparan-induced PRL secretion which is inhibited by the nifedipine, an L-type Ca2++ channels blockers. Mastoparan effects on casrrot cell plasma membrane polyphosphoinositide phosdpholipase C[142] (Table 1).

Philanthotoxin (PhTX) is a neurotoxic constituents of the paratylic venom of the digger wasp, *philanthus triangulum.* PhTX inhibits glutamate receptors of insects muscles mostly as a channels blockers, thereby producing muscle paralysis.[143] Its structure activity relationship studies reveal that shortening the chain length reduced potency. It was found increased by a bulky anchoring group with moderate hydrophobicity at the end of the polyamines chain. Philanthotoxin-433 (PhTX-443) is a non-compititive channels blockers found in the venom of the wasp *Philanthus* and interects with nAChRs similar to polyamines toxins.[144] PhTXs not only inhibit different subtypes of the glutamate receptors of the insects and mammalians N-methyl-D-Aspartate receptors, but also nicotinic receptors of insects vertebrates. Due to this property PhTXs can be used as a pesticide.[143] Similarly, tetrodotoxins are neurotoxins which blocks voltage gated sodium ion channels of purkinje neurons produce resurgent currents with repolarization. Its results from relief of an open-chennals block that terminates currents flow at positive potentials.[145] Similarly, β-PMTX increased resurgent current in wild type neurons and induced resurgents currents in med neurons.[145] Phylanthotoxins (PhTX) possess varying structures and interacts with the two receptors nicotine acetylcholine (n Ach-R) and N-methyl-D-Aspartate receptors bind these two sites: external polyamines binding sites and a channels binding site.[146] The nicotinic acetylcholine receptors (nAChRs)

and glutamate receptors are ligand-gated cation channels composed of five separate polypeptides chains. Mastoparans a wasp venom toxins, effects on intracellular free Ca2++ concentration in human astrocytoma cells. Mastoparans inhibits phosphoinositide hydrolysis in human astrocytoma cells.[119] Similarly, AvTx-7 isolated from the *Agelaia vicina* (Hymenoptera: Vespidae) wasp venom effects synaptosomal glutamate uptake and release.[133]

Melettin and mastoparan are amphiphilic peptides found in hymenopterid insects venom gland. Melettin stimulate calcium influx some 20-fold more potently than mastoparans. Melettin stimulate both breakdowns of phosphoinositides (PI) by phospholipase C to yield inositol phosphates and hydrolysis of phospholipids by phospholipase A2 to released arachidonic acid (AA).[127] The influx of calcium elicited by melettin or mastoparans is completely or nearly blocked by inorganic calcium channels blocker (Co++, Mn++, Cd++), these inhibits melettin-induced PI breakdown and AA release and mastoparans-induced PI breakdown.[127] PTX-443, the synthetic product of the wasp toxins, delta-philanthotoxins, blocks the open, glutamate gated ion channels of the locust muscles[107] (Table 1).

Venoms from several arthropods are recognized as useful sources of bioactive substances, such as peptides, acylpolyamines, and alkaloids, which show a wide range of pharmacological effects on synaptic transmission.[147] More specifically, delta-Philanthotoxin, was found a potent a semi-irreversible blocker of ion-channels. Pompilidotoxins (PMTXs, alpha and beta) are small peptides consisting of 13 amino acids purified from the venom of the solitary wasps *Anoplius samariensis* (alpha-PMTX) and Batozonellus maculifrons (beta-PMTX)[100], These toxins showed voltage-gated sodium channel isoform-specific effects and are known to facilitate synaptic transmission in the lobster neuromuscular junction. These toxins instantly slowly did sodium channel inactivation. PMTXs mainly beta-PMTX, alpha-PMTX and four synthetic analogs showed sodium current inactivation in seven mammalian voltage-gated sodium channel (VGSC) isoforms and one insect VGSC (DmNa(v)1). Binding shows complex and intriguing behavior of VGSCs in response to PMTXs.).[100] Similarly, Polybia-MPII (INWLKLGKMVIDAL-NH2), a mastoparan isolated from the crude venom of the swarming wasp *Polybia paulista*, binds to motor end plates that are identified by the positive labelling of acetylcholine receptors by TRITC-alpha-bungarotoxin co-labelled with anti-synaptophysin antibody. No doubt mastoparan Polybia-MPII is a minor neurotoxin that shows neurotoxic activity and is unlikely to be of clinical significance.[101] Similarly,

wasp *Ampulex compressa* injects venom directly into the prothoracic ganglion of its cockroach host to induce a transient paralysis of the front legs. More specifically, Dabsylation of venom components produces transient block of synaptic transmission at the cercal-giant synapse and block efferent motor output generates in the prothoracic ganglion, it mimics effects produced by injection of whole venom. Contrary to this whole venom evokes picrotoxin-sensitive chloride currents in cockroach central neurons, consistent with a GABAergic action.[102] Ampulex utilizes GABAergic chloride channel activation as a strategy for central synaptic block to induce transient and focal leg paralysis in its host.[103] (Table 1).

Wasp venom immunotherapeutics

Venoms from several arthropods are recognized as useful sources of bioactive substances, such as peptides, acylpolyamines, and alkaloids, which show a wide range of pharmacological effects on synaptic transmission. Diagnosis of insect sting allergy and prediction of risk of sting anaphylaxis are often difficult because tests for venom-specific IgE antibodies have a limited positive predictive value and do not reliably predict the severity of sting reactions. Component-resolved diagnosis using recombinant venom allergens has shown promise in improving the specificity of diagnostic testing for insect sting allergy. Basophil activation tests have been explored as more sensitive assays for identification of patients with insect allergy and for prediction of clinical outcomes. Measurement of mast cell mediators reflects the underlying risk for more severe reactions and limited clinical response to treatment. Measurement of IgE to recombinant venom allergens can distinguish cross-sensitization from dual sensitization to honeybee and vespid venoms, thus helping to limit venom immunotherapy to a single venom instead of multiple venoms in many patients. Basophil activation tests can detect venom allergy in patients who show no detectable venom-specific IgE in standard diagnostic tests and can predict increased risk of systemic reactions to venom immunotherapy, and to stings during and after stopping venom immunotherapy. The risk of severe or fatal anaphylaxis to stings can also be predicted by measurement of baseline serum tryptase or other mast cell mediators.[148] Hymenoptera venom toxins show various biological activities such as antimicrobial[149], anticancer [150] and antitumor activity both *in vitro* and *in vivo*. It also causes apoptosis, tissue necrosis and lysis of tumor cells and finally inhibits the tumor growth.[151] These toxins also function as antibiotics[152] and are used by the animals in self-protection.[153] The venom immunotherapy (VIT) is commonly used for preventing allergic reactions to insects sting in the people who

have had a sting reaction. It is highly efficacious in preventing anaphylactic sting reaction, regarding patient selection.[154] The venom immunotherapy using extracted insects venom to be an effective therapy for preventing future allergic reaction to insect's stings, which can improve quality of life.[155] *Vespa tropica* (VT) venom and its isolated peptides showed anti-inflammatory potentials. Both whole venom and its peptides (Vt1512 and Vt1386) effects on lipopolysaccharide (LPS) challenged BV-2 murine microglial cells. Purified peptides from crude venom exhibited potential anti-inflammatory properties. Further, whole venom was found to be targeting Akt and p38 MAPK pathways, leading to suppressed NF-κB phosphorylation in LPS challenged BV-2 cells. VT venom possesses anti-inflammatory properties and can be further explored for their therapeutic potential in treating various inflammatory conditions of the central nervous system (CNS).[156](Table 1).

The diagnostic work-up of wasp and bee venom allergy and treatment by specific venom-immunotherapy (VIT) is well established and suspected allergy is most often confirmed by skin testing. *In vitro* diagnostic by the specific IgE has still a diagnostic gap of up to 20-30% particularly when performed more than 1 year after the last sting reaction. Therefore, additional *in vitro* methods are desirable to make *in vitro* testing as reliable as in vivo testing. Secondly, despites great progress in understanding the mechanism of VIT, there is still no *in vitro* test which predicts whether a patient has been successfully tested by VIT, i.e., whether a patient will or will not have a systemic reaction in the case of a future sting. Basophils are often used as target cells for in vitro assays to detect IgE-mediated sensitization.[157] Although anti-IgE antibody (Ab) therapy found effective in patients with bronchial asthma, but its effects are used in the prevention of wasp-venom anaphylaxis. The anti-IgE.Ab treatment is an effective preventive measure against wasp-venom induced anaphylaxis. It is estimated that upto 5% of the general population suffer from potentially life-threatening systemic reactions after hymenopteran sting (Table 1)

Anaphylaxis due to hymenoptera stings is one of the most severe clinical outcomes of IgE-mediated hypersensitivity reactions. IgE-reactive a 200 kDa protein isolated from of *Apis mellifera* and *Vespula vulgaris* venom shows allergic reactions and systemic side effects.[158] But it is a potential candidate for overcoming the life-threatening resistance of pathogenic microorganisms to classic antibiotics.

Anti-IgE Ab has demonstrated its effectiveness against asthma, allergic rhinitis and food allergy in human and in animal model. The first time that anti-IgE Ab suppresses the onset of

Anaphylaxis.[159] Early clinical tolerance occurs in the face of persisting antigen-specific IgE and can be mediated at least in part, by the passive transfer of IgG derived from previously exposed, but tolerant individuals.[160] Thus specific IgG blocking antibodies have been implicated in the generation of early clinical non-responsiveness and may be induced through local and systemic IL-10 derived from regulatory T-cells and other cells.[161] Each year in the UK there are between two and nine death from anaphylaxis caused by wasp and bee venom. Anaphylactic reaction to bee and wasp venom are a medical emergency, necessitating immediate treatment with drugs, oxygen and fluid to decrease the patients response to the venom and support breathing and circulation, to the most severe systemic allergic reactions is referred to as anaphylaxis, which is characterized by features such as low blood pressure, bronchospasm (asthma like response) and laryngeal oedema. Venom immunotherapy (VIT) is used to detoxify and neutralize the effect of venom in patients. VIT consist of subcutaneous injections of increasing amounts of purified bee and wasp venom extracts.[162] With respect to its pathogenesis, hymenoptera-toxin anaphylaxis is a typical immediate-type allergic reaction. Specific IgE antibodies directed against components of the toxins mediated the activation of mast cells and basophilic granulocytes, leading to release of mediators that cause the acute manifestation of diseases. The main causes of death due to anaphylaxis are airway obstruction and cardiovascular failure. Myocardiac infarction, stroke and thrombotic events can cause permanent morbidity.[163] Specific immunotherapy (SIT) is the treatment of first choice for the patient who has had a systemic immediate reaction to a Hymenoptera stings. SIT is highly useful and efficacious for patient stung with bee and wasp venom.[164] The standard maintenance dose of 100µg of insects venom protects about 75% to 95% of persons so treatment from a further episode of sting anaphylaxis[68] if the treatment is ineffective at first, it nearly always succeeds when the maintenance dose is increased. Toxins also show non-specific cytolytic activity as well as their rapid degradation and excretion when injected in the blood. Therefore toxin lytic peptides can be used for tumor suppression and for spatiotemporal control of tumor cells proliferation (Moreno et al., 2014).[165] Many low molecular weight compounds isolated from wasp venom shows dose-dependent anticonvulsant effects in animals.[45] Toxins induce intracellular histamine release that may also use for tumor suppression.[166]

Vespa velutina, the most common wasp species native in China contains allergens usually regarded as the major lethal factors of wasp stings. Venom of *Vespa velutina* was identified, implying that toxins reactions and allergic effects are envenoming strategy for the dangerous

outcomes.[167] The bee and wasps venom are therapeutic effects of these toxins in numerous diseases, the three most characterized peptides, namely melittin, apamin and mastoparans.[168] The patients with a history of anaphylaxis to hymenoptera venom and possitive specific IgE have shown a correlation between elevated tryptase levels and two clinical situations, systemic mastocytosis and an increased risk of reactions to venom immunotherapy or hymenopteran stings. Other clinical scenarios could explain elevated tryptase levels.[169] Specific immunotherapy is a very effective and well tolerated therapeutic option in patients with hymenoptera venom allergy. Many patients can be successfully treated, and severe side-effects are rarely seen. In most cases, local swelling of the injection site is noticed, whereas systemic reactions are uncommon. No reliable biomarkers to prove the positive response to the specific immunotherapy have been validated. But on the other hand failure of the venom immunotherapy can be repeated. Since, the IgE-antibody omalizumab have been licensed for different indications, nee therapeutic options is available.[170]

Hymenoptera stings give rise to anaphylactic reactions in1.2 to3.5% of the populations. The risk of repeat anaphylaxis following subsequent stings is greatly reduced through immunotherapy with the culprit venom. In patients with no sensitization in skin or serological tests but with a convincing history of the insects stings anaphylaxis, the increased sensitivity of a basophils to activation test may deliver crucial evidence of the venom sensitization. The value of the basophil activation test may be further improved using specific markers allergens. The diagnosis of allergy to paper wasps (Polistinae) and white-faced hornets (*Dolicho vespula)* remains problematics as they show partial cross-reactivity to wasp venom and specific marker allergens particular to these.[171] The parasitoid jewel wasps uses cockroaches as live food supply for its developing larva. The adult wasp stings a cockroaches and injects venom directly inside its brain, turning the pray into a submissive "Zombie" we characterized the sensory arsenal on the wasp's stinger that enables the wasp to identify the brain target inside the cockroach's head, and electron microscopy study of the stinger reveals.

Evolutionary significance of Channel Blockers

From an evolutionary point of view, the presence of anti mammal toxic effect next to anti-insect toxicity is a progressive adaptation of the venomous secretion of putative action wasps. Selective anti-insect toxins are not found in the wasps and only a few toxins have bi-specificity. Most of the toxins in arthropods were found activity against enzymes and sodium channel receptors of mammals and few insects only.[172, 173] Arthropods possess ion channel

blockers [174] which are highly diversified according to the survival of venoming species.[175] The mode of conversion of old world toxins (alpha type) into new world beta type toxins remains unsolved to date. Animal toxins have structural and evolutionary relationships between different animal groups. Due to environmental changes, site-specific mutagenesis and rearrangements diverse toxin groups are evolved. Their toxicity depends on the amino acid residues present in the active site region and topological folding occur due to temperature sensitivity and elements of earth. Modifications in active site region led to the structural and functional diversification of toxins and established different biologically activity during the long course of time. From the evolutionary point of view the voltage-gated channels are cellular adaptive structures, which display affinity to the toxin as ligands. These ion channels behave as specific gates and help in ionic conduction through the membrane. Their opening and closing depend on electrical potential generated by specific stimulus. The cells devoid of these channels are more primitive to those bearing these channels. For formation of physiological electrochemical gradient these channels depend on ion permeability all along both sides of nerve membrane. Permeability of ions also depends on their size, types of channel and electric discharges. These channels solely depend for physiological action on electrochemical gradient formed on both sides of the membrane [176] all these channels are found in host cells or in prey.

The divergence in evolution has generated different interrelated clusters of toxins within the families/genera. Evolution of diverse defense mechanism indicates that toxins, proteins, co-enzymes are the molecules which provide protection to the animals. Overall diversification in toxins depends upon the types of tissues and environment of the animals. In different prey/ host animals pores of channels get blocked and ionic conduction inhibited for the protection against toxin action. It is one of the most highly significant molecular defense mechanism, which has developed during long evolution. Similar mechanisms have been observed in other animal groups on toxin diversification and mechanism of their physiological action. There are very few venom toxins, which impart high toxic effect on both mammals and insects; their toxic effects are very weak in comparison to anti-insect toxins inhibitors. It is well known that wasp toxins are ion channel blockers. These also show cytolytic mainly hemolytic activity and bound to neuronal receptors. Small toxins peptides, which have both beta and alpha type structures, show bifurcation of toxins into two groups. When an amino acid with non-charged polar group responsible for non-target of channel binding is

transformed into a basic amino acid the polypeptide becomes toxic. The other types of toxins are high-grade killers containing highly charged basic amino acids in their α-helical region.

Table 1: showing important wasp venom toxins, their nature and physiological effect in animals.

S.N	Species name	Nature of toxin	Biological effects
1.	Vespa tropica	Chemotactic peptides, antimicrobial peptides, natural host defence peptides.	Antimicrobial activity activate cell-mediated antiviral immune response.
2.	Vespa orientalis	Crude venom	Inhibits the growth of gram (+) and gram (-) bacterial cells.
3.	Vespa affinis	Allergic proteins	Local reaction, IgE mediated anaphylacxis, systemic reaction.
4.	Polibia paulista	Common peptides	Defensive tools to protect the colonies of the insects against the attacks of the predators.
5.	Nasonia vitripensis	Polistine peptides, common peptides, 128 genes.	Reduce thoil group of cysteine residue do not form disulphide briedge, promote programmed cell-death, elicited detoxificatyion responses, and induced immune responces.
6.	Polibia polista	Phospholipase, hyluronidases, mastoparans	Genotoxic, mutagenic and damages in the genetic material.
7.	Polibia polista	cationic peptides (polibia MP-I)	Antifungal activity
8.	Vespa flavitarsus	Hornetin protein	Hemolytic activity in RBCs and presynaptic neurotoxicity.
9.	Polibia scutellaris	Hypoallergic varients	Antigen 5 allergens, hypoallergic varients.
10.	Anopolis samariensis	Alpha-PMTX-13 amino acid peptides	Both excitatory or inhibitory synaptic transmittion.
11.	Vespa valgeris	Phospholipase A-, hyluronidases, and antigen 5	Severe local swelling accompanied by fever
12.	Vespa germanica	Phospholipase A-, hyluronidases, and antigen 5	Severe local swelling accompanied by fever
13.	Chelonus inanitis	Potentially lineage-specific protein (hyluronidases and allergen 5)	Parasitoids from encapsulation by hosts nutritional physiology and induce a developmental arrest in the prepupal stage.
14.	Agelaia pallipes pallipes	Cationic peptides, antimicrobial peptides-crude venom	Antimicrobial activities, antitumors drugs and inhibits the growth of gram positive and gram negative bacterial cells.
15.	Vespa crabro (Linaeus)	Crude venom peptides	Local reaction, swelling etc.
16.	Polibia polista	Wasp kinins, chemotactic components, mastoparans peptides	Pain, oedema formation, heamolysis,chemotaxis and mast cell degranulation.
17.	Nasonia vitripensis	Steroids,cytokine IL-1β	Alters the expression of some drugs targets, NF-κβ pathway can be effected.
18.	Vespa velutina	Hemostasis-imparing toxins	allergic effects and toxic effects.

19.	Agenlaia pallipes pallipes	Protonectin peptides	Promotes mast cell degranulation activities.
20.	Vespa flavitersus	Hornetin protein peptides	Hemolytic activity in red blood cells and presynaptic neurotoxicity.

CONCLUSION

Wasp toxins are useful sources of bioactive substances, such as peptides, acylpolyamines, and alkaloids, which show a wide range of pharmacological effects on synaptic transmission. Wasp venom toxins cause sudden inflammation in body cells with a severe pain and do massive inhibition of axonal transmission in neurons. The activity of ATP driven Na^+-K^+ ATPase pump, which plays a key role in maintaining cell volume and intra cellular ionic composition specially Na^+ and K^+ gradients is also affected by venom toxins. Physiologically animal toxins are highly active as they block various channels and breach the normal barrier for free movement of molecules across cell membrane. Wasp venom toxins do sodium current inactivation in mammalian and voltage-gated sodium channel (VGSC). Venom toxins inhibit the calcium influx via voltage dependent as well as NMDA mediated calcium channels and reduce excitability of muscles and skeletal tissues. Several wasp venoms contain natural polyamine amides, which act as noncompetitive inhibitors (NCIs) on the nicotinic acetylcholine receptor (nAChR). Wasp venom toxins bind to K channels and inhibit maintaining resting membrane potential and regulating excitability in many cell types. Wasp toxins are potent voltage-dependent blockers and glutamate receptors inhibitors which work as potential insecticides. Venoms from several wasp species possess strong allergens which after infliction induce allergy. It will need venom-specific IgE antibodies for immunotherapy mainly for neutralization of venom generated allergic and toxic effects. Wasp venom toxins can be used as proves and extensively used as antibiotics agents against multidrug resistant microbes. Venom toxins can work as alternative medicine to replace synthetic antimicrobial agents resistant to bacteria. Allergen sensitivity should be used to identify patients with a high risk for severe side-effects. Wasp natural antimicrobial peptides (AMPs) can provides structural-functional information for designing peptides antibiotics with therapeutic potential.

Abbreviations: iGlu =ionotropic glutamate MSCs=mechanosensitive channels, MPs= membrane-active peptides, PhTXs =philonthotoxins ,VGCC= voltage-gated sodium channel blockers , AMPs= antimicrobial peptides , CNS =central nervous system, N-methyl-D-aspartate (NMDA), GABA= gamma amino butyric acid, nAChR =nicotinic acetylcholine

receptors (nAChR), IGRs =ionotropic glutamate receptors, NMDAR= N-methyl-d-aspartate (NMDAR), AMPARs =isoazol propionic acid receptors, WVA= wasp venom allergy, VIT=Venom immunotherapy.

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