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A Review

Pharmacological and therapeutic potential of honey bee antimicrobial peptides

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Honey bees (Apidae: Apini) and stingless bees (Apidae: Meliponini) act as the main pollinators for many wild and cultivated tropical plants, playing a vital role in the ecology, economy, and culture. Honey bees and stingless bees are one of the major sources of antimicrobial peptides/proteins (AMPs) synthesized in fat bodies and blood cells of bees. Bee AMPs are a class of small peptides having amino acid residues between 9 and 340, classified based on source, activity, structural characteristics, and amino acid-rich species. AMP's have a wide range of inhibitory effects against bacteria, fungi, parasites, and viruses. Four antimicrobial peptide families, *i.e*., apidaecins (proline-rich), abaecin (proline-rich), hymenoptaecin (glycine-rich), and defensin (cystine-rich) are synthesized in the haemolymph, signifying a broad spectrum of antimicrobial activity. Jelleines (I-IV), royalisin, apisimin (serine-valine-rich peptide), 10 HDA, apalbumin, and apisin, which are present in royal jelly, have antimicrobial, mast cell degranulating, and hemolysis activity. Bee venom also contains several bioactive peptides, such as apamin (leucine-cystine-rich), melittin (leucine-alanine-rich), melectin (lysine-rich), adolapin, secapin (proline-rich), and tertiapin (cystine-lysine-rich). Currently, AMPs databases are displaying an essential role in exploration, identification, characterization, and annotation. Several AMPs databases (CAMP, DRAMP, APD, InverPep, LAMP, ADAPTABLE, ADAM, AntiBP, AMPer, AVPpred, EFC-FCBF, and class AMP) are open-access resources that have been developed to enhance research on antimicrobial peptides. Bee immune responses are composed of a multifaceted group of individual immune mechanisms and special types of behavioral adaptations. Given the importance of drug discovery from honey bee AMPs, this review is aimed at providing an exhaustive screening of the AMPs detected in honey and honeybee products and their classification, databases, computational tools, physicochemical properties, signaling pathways, pharmaceutical and clinical uses, application status, prospects, and problems to be solved.

Keywords: AMPs, Databases, Honey bees, Signaling pathways, Therapeutic applications

Introduction

Honey bees and stingless bees, act as one of the major pollinators in wild forest and agriculture systems, are indispensable for conserving ecological biodiversity, global ecological stability, productivity and economy[1]. Besides, both honey bees and stingless bees are capable to produce different types of honey, royal jelly, bee wax, propolis, bee bread and bee venom based on diverse floral resources visit and can yield significant contributions to human society (Fig. 1). Nevertheless, several factors seemingly lead to bee depopulation and colony/brood loss events, including pathogens (parasites, fungi, bacteria and viruses), ecosystem alteration or loss, and the use of pesticides and antibiotics intimidate wild plant diversity, terrestrial ecosystem stability, crop production, global food supply, and human welfare[2]. The immune system of bees is capable of changing its defence mechanisms, so it is essential to understand how it works in order to examine how it reacts to various pathogenic and stressful situations that affect the health and behaviour of bee colonies (Fig. 1).

Bee immunity serves as an example of the superorganism theory since it relies on both individual and colony-level defence mechanisms to protect bees from infections and stressful situations[3]. Additionally, bees' social immunity—a network of behavioural, physiological, and organizational responses that prevents the admission, establishment, expansion, and transmission of parasites and microbial diseases in the colony—supplements their physiological immune systems. Bee immune responses are comprised of distinctive categories of behavioral adaptations, evolutionary conserved defense strategies (cellular and humoral responses) and assemblage of multifaceted discrete immune mechanisms that afford

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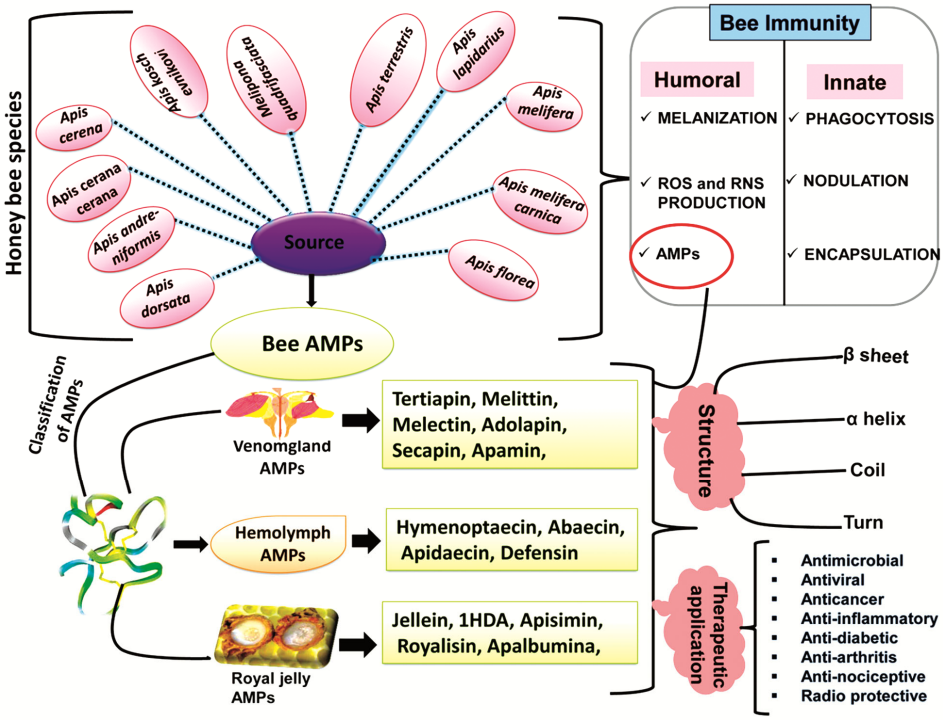

Fig. 1 — Sources of antimicrobial peptides from honey bee species, classification and their role in bee health

immediate responses against different pathogens and biotic and abiotic stressors[4]. Defense machinery in bees is entirely built on an effective innate immune system, which permits a broad and speedy response to different invading pathogenic and parasitic organisms[5]. Bees' immunity consists of the three levels of defense strategies, *i.e.*, 1) first line of defense-physical barriers (exoskeleton, cuticle, tracheal tubes, mucous membranes, intestinal wall, peritrophic membrane *etc.*), 2) cell-mediated immunity (phagocytes and haemocytes), and 3) cell-free humoral immunity, an intricate grid of intracellular signaling pathways precede to instigation of diverse of humoral effectors as supplement system fractions, acute phase proteins, thioester linkage proteins, melanization, coagulation proteins, antimicrobial peptides (AMPs), natural antibodies, and the various cytokines that modulate immune response[6] (Fig. 1). The immune system of bees is clustered into 17 families and analogous to dipteran insects. Bees' immune genes are shared by *Drosophila* and *Anopheles* to the tune of 33.33%. For odour recognition and particular genes for floral resource sensing, social behaviour, and organization, bees have more protein-coding genes than other insects. The significance of social defences (social behaviour and organization) in the bee colony and their propensity to disease from a specific set of pathogens, which have evolved to make up for less expenditure in physiological immunity and reduction of selection pressure for a stronger individual immune defence, may be revealed by the inherent decrease in the number of immune genes in bees[6].Humoral effectors are playing a major role in cellular immune responses in bees. Fat body is the major resource for effectors synthesis followed by hemocytes, epithelial cells and salivary glands. Haemocytes (prohemocytes, clot haemocytes, granular cells and oenocytoids, pro-leukocytes, picnonucleocytes, adipoleukocytes, spherukocytes, granulocytes, macronucleocytes, microleukocytes, and spindle-type cells) are not only modulating the process of nodulation, encapsulation and phagocytosis to combat the invading pathogens but also involved in the signal transduction pathways and aided for the production of AMPs, regulatory role in activation hemolymph coagulation and melanization, and reactive oxygen and reactive nitrogen species (ROS and RNS) production along with the fat body cells[7,8] (Fig. 1).

Innate immune system *vs* social immunity

Innate immunity is playing a prospective role in sting and stingless bees to modulate mortality as well as pathogen transmission among colony members.

Immunity in bees characterizes the "superorganism concept" signifying the presence individual and colony level immune defense system to support survival of both the individual bee as well as the whole bee colony. The decrease in immune genes in bees demonstrates the impact of colony-level defenses than individual immune defense[3]. Once defense mechanisms depend on collective activities to check, minimize or eliminate pathogens and parasites are known as 'social immunity'. The social immunity in bees is categorized into two gradients based on their functional mechanisms as the types of immune activation (constitutive *vs* inducible) and the levels of defense (individual *vs* colony) (Fig. 1). Constitutive defenses are the first line of defense, prophylactic, always exist, and no alteration when individuals or colonies are exposed to abiotic and biotic stresses while inducible defenses are synchronized or activated by pathogens/parasites exposure. Constitutive group behavioral defenses of colony level immunity include the traits of task allocation, external use of glandular compounds, pharmacophory (propolis and resin), trophallaxis of antimicrobials and self-produced/environmentally collected compounds (defensin 1 and glucose oxidase), whereas phenoloxidase cascade is in constitutive defenses of individual immunity. Hygienic behavior, social fever, social exclusion and self-removal, brood cannibalism and allo-grooming traits are highlighted as inducible defenses of social immunity, while self-grooming, AMPs and pharmacophagy are inducible defenses of individual immunity[7,8] (Fig. 1).

Antimicrobial peptides (AMPs)

AMPs are fundamental components of bee innate and cellular immune system and synthesized in response to immune challenge by pathogens and parasites in the fat body or haemocytes and secreted successively into the hemolymph. AMPs are playing a significant role as host defense peptides in honey bees and stingless bees (Fig. 1). It is one of the ancient evolutionary mechanisms of bees which can be produced and transported to the site of infection within the short span of interval[8].

Bee AMPs are small cationic (positively charged), amphiphilic (hydrophilic and hydrophobic) and coiled and α-helical peptide molecules comprising of 9-340 amino acids, that have been identified in honey bees (*Apis cerana*, *A. cerana cerana*, *A. cerana japonica*, *A. mellifera*, *A. mellifera carnica*, *A. terrestris*, *A. koschevnikovi*, *A. andreniformis*, *A. lapidaria*, and *A. dorsata*) and stingless bees (*Melipona quadrifasciata* and *Heterotrigona itama*) (Fig. 1 & Table 1). A total of 3257 AMPs have been recognized till date across phyla as per the APD3 database[9] including bacteria (365), archaea (5), protists (8), fungi (22), plants (360), and animal (2414) origin (Table 2). Until now, the APD3 database is describing 323 insect-derived AMPs[9] wherein only 15 AMPs (apidaecin, abaecin, hymenoptaecin, defensin, royalisin, jelleine, apisimin, 10 HDA, apalbumina, apamin, melittin, melectin, adolapin, secapin and tertiapin) reported for honey bees and stingless bees (Figs 1, 2 and Tables 1-4). Four antimicrobial peptide families, *i.e*., apidaecins (proline-rich), abaecin (proline-rich), hymenoptaecin (glycine-rich) and defensin (cystine-rich) are synthesized in the haemolymph signifying a broad spectrum of antimicrobial activity while jelleines (I-IV), royalisin, 10 HDA, apalbumina and apisimin (serine-valine-rich peptide) present in royal jelly having antimicrobial, mast cell degranulating and hemolysis activity. Bee venom also contains several bioactive peptides like apamin (leucine-cystine-rich), melittin (leucine-alanine-rich), melectin (lysine-rich), adolapin, secapin (proline-rich), and tertiapin (cystine-lysine-rich) (Figs 1,2 and Tables 1-4). Details about honey bee antimicrobial peptides, their physico-chemical properties and available databases[10] are shown in (Tables 1 and 2).

Apidaecin (apidaecin-type peptides)

The smallest and most abundant class of proline-rich peptides (18–20 amino acid residues) is known as apidaecin, and it is generated by stingless bees and honey bees. Apidaecin-like peptides from insects are often shorter than those from mammals[11]. Four isoforms of apidaecins [HbIa (**GNNRP**VYIPQ **PRPPHPR**I), HbIb (**GNNRP**VYIPQ**PRPPHPR**L), HbII (**GNNRP**IYIPQ**PRPPHPR**L) and HbIII (**GNNRP**IYISQ**PRPPHPR**L)] were characterized from the haemolymph of *Escherichia coli* infected *A. mellifera*. It contains conserved and variable regions accountable for antimicrobial activity and conferring microbial specificity, respectively. Apidaecin is protective against gram-negative bacteria by two different mechanisms: 1) inhibition of the DnaK protein (bacterial chaperone network), which blocks ATPase activity and eliminates its capacity to promote chaperone-assisted protein folding and ribosomal biogenesis; and 2) stop codon-dependent

Table 1 — Details about honey bee antimicrobial peptides and their physico-chemical, pharmacological and therapeutic properties

Physico-chemical properties of honey bee antimicrobial peptides

Sl. No	Peptide name	Antimicrobial Peptide	Length of AMP	Molecular Weight (kDa)	References
		Hemolymph			
1	Abaecin	MKVVIFIFALLATICAAFAYVPLPNVPQPGRRPFPTFPGQGPFNPKI KWPQGY	53	5903.09	(10)
2	Apidaecin	MKNFALAILVVTFVVAVFGNTNLDPPTRPTRLRREAEPEAEPGNN RPVYIPQPRPPHPRLRREAEPEAEPGNNRPVYIPQPRPPHPRLRREA EPEAEPGNNRPVYIPQPRPPHPRLRREAEPEAEPGNNRPVYIPQPRP PHPRI	144	16538.80	
3	Defensin	MKIYFIVGLLFMAMVAIMAAPVEDEFEPLEHFENEERADRHRRVT CDLLSFKGQVNDSACAANCLSLGKAGGHCEKGVCICRKTSFKDL WDKRFG	95	10717.45	
4	Hymenoptaecin	MKFIVLVLFCAVAYVSAQAELEPEDTMDYIPTRFRRQERGSIVIQG TKEGKSRPSLDIDYKQRVYDKNGMTGDAYGGLNIRPGQPSRQHA GFEFGKEYKNGFIKGQSEVQRGPGGRLSPYFGINGGFRF	129	14492.42	
		Major Royal Jelly			
1	Jellin	EPFKISIHL	9	1083.30	(10)
2	Apisimin	MSKIVAVVVLAAFCVAMLVSDVSAKTSISVKGESNVDVVSQINSL VSSIVSGANVSAVLLAQTLVNILQILIDANVFA	78	7946.39	
3	Royalisin/ Defensin	MKIYFIVGLLFMAMVAIMAAPVEDEFEPLEHFENEERADRHRRVT CDLLSFKGQVNDSACAANCLSLGKAGGHCEKGVCICRKTSFKDL WDKRFG	78	7946.39	
		Bee Venom			
1	Tertiapin	ALCNCNRIIIPHMCWKKCGKK	21	2460.09	(10)
2	Melectin	GFLSILKKVLPKVMAHMK	18	2040.64	
4	Apamin	MISMLRCIYLFLSVILITSYFVTPVMPCNCKAPETALCARRCQQHG	46	5223.37	
5	Melittin	MKFLVNVALVFMVVYISYIYAAPEPEPAPEPEAEADAEADPEAGI GAVLKVLTTGLPALISWIKRKRQQG	70	7584.86	
6	Secapin	MKNYSKNATHLITVLLFSFVVILLIIPSKCEAVSNDMQPLEARSAD LVPEPRYIIDVPPRCPPGSKFIKNRCRVIVP	77	8664.35	

Pharmacological and therapeutic properties of antimicrobial peptides derived from honey bees

Sl. No	Antimicrobial peptides	Pharmacological and therapeutic properties	References
1	Defensin-1	Antibacterial activity	(27)
2	Apalbumin	inhibition of ROS release, NO production, phagocytosis, and the production of TNF-α	(28)
3	Melittin	Anticancer, anti-tumor, anti-angiogenesis, anti-fungal, anti-parasitic, anti-microbial, anti-arthritic, anti-inflammatory, anti-psychotic, anti-atherosclerosis, anti-bacterial, anti-viral, neuroprotective and anti-multidrug resistant	(20, 30, 32)
4	Apamin	Blood-brain barrier drug-delivery shuttles, anti-fungal, anti-viral, anti-inflammatory, analgesic and neurodegenerative disorders	(19, 22, 23)
5	Jelleins	Antimicrobial activity	(16)
6	Royalactin	Growth and development, and Antiaging	(35)
7	Royalisin	Antibacterial, antifungal	(36)
8	10-HDA	Immunomodulatory activities, antiulcer, anti-inflammatory, and neurodegenerative disorders	(37 – 40)
9	Adolapin	Anti-arthritic, analgesic, anti-inflammatory, antipyretic, anti-nociceptive	(22)
10	MCD peptide	Anti-inflammatory	(34)

inhibition of translation by apidaecin[11,12] (Figs 1 & 2, and Tables 1-4).

Abaecin

It is one of the largest proline-enriched cationic broad-spectrum antibacterial peptide (34 amino acids and no cysteine residues) having 29% of proline residues (10 nos.) which distributed uniformly causing no α-helical conformation[11] (Figs 1 & 2, and Tables 1-4).

Apisimin

Apisimin is a small, serine-valine-rich peptide isolated from the royal jelly of *A. mellifera*. It is an acidic peptide containing 54 amino acids and devoid

Table 2 — Databases available for antimicrobial peptides

Sl. No.	AMPs database	Websites	AIM and objective of the databases	References
1.	NCBI	The National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/	NCBI consists of 769131 AMPs, of which 93213 from *Streptococcus pneumoniae*, 66764 from *Escherichia coli*, 42212 from *Salmonella enterica*, 29225 from *Staphylococcus aureus*, 25851 from *Klebsiella pneumoniae*, and 511866 from all other taxa.	(10)
2.	Uniprot	Universal Protein Resource is a comprehensive resource for protein sequence and annotation data. https://www.uniprot.org/	Uniprot database having 53357 no of antimicrobial peptide across the flora and fauna. Sequence length ranged as 1 - 200 (16,184 numbers), 201 - 400 (17,547 numbers), 401 - 600 (11,646 numbers), 601 - 800 (3,630 numbers) and >= 801 (4,350 numbers)	
3.	$CAMP_{R3}$	Collection of Anti-Microbial Peptides http://www.camp3.bicnirrh.res.in/	This database contain 8164 AMPs sequences, 757 structure, 2083 patented AMPs.	
4.	DRAMP	Data repository of antimicrobial peptides http://dramp.cpu-bioinfor.org/	AMPs sequences, structures, activities, physicochemical, patent, clinical and reference information. 22209 entries, 5841 of which are natural and synthetic AMPs, 16110 patent AMPs and 77 AMPs in drug development (preclinical or clinical stage).	
5.	APD3	Antimicrobial Peptide Database https://wangapd3.com/main.php	Contains 3257 antimicrobial peptides from six different kingdoms 365 from bacteria, 5 from archaea, 8 from protists, 22 from fungi, 360 from plants, and 2414 from animals.	
6.	InverPep	Database for AMPs from invertebrates https://ciencias.medellin.unal.edu.co/gruposdeinvestigacion/prospeccionydisenobiomoleculas/InverPep/public/home_en	702 peptides with identification code, source (phylum and species), name, sequence, length, secondary structure, molar mass, charge, isoelectric point, hydrophobicity, Boman index, aliphatic index, percentage of hydrophobic amino acids, target organisms, experimental verification and external literature.	
7.	LAMP2	A database linking antimicrobial peptides http://biotechlab.fudan.edu.cn/database/lamp/	LAMP2 currently has 23253 AMP sequences, including 7824 natural and 15429 synthetic peptides.	
8.	DBAASP	The Database of Antimicrobial Activity and Structure of Peptides http://dbaasp.org.	DBAASP containing information about 20523 peptides.	

of cysteine, methionine, proline, arginine, histidine, tyrosine, and tryptophan residues. It contains 18.5% of valine and 16.7% of serine with only one phenylalanine residue. Molecular mass of mature apisimin is 5540.4 Da and was found in the high molecular weight fraction of royal jelly. The primary structure of apisimin is KTSISVKGESNVDVVSQIN SLVSSIVSGANVSAVLLAQTLVNILQI LIDANVFA-NH_2. The major secondary structural elements in apisimin is α-helical (34%) followed by β-sheet (20%), β-turn (11%) and random coils (30%). High amount of apisimin mRNA and apalbumin-mRNA (MRJP-1) was found in the heads of honeybees and are synthesized during the whole life span of honeybees. This indicates that both the AMPs are associated with larval development and learning and memory of both nurse and forager honeybees. Protein sequence of apisimin not showing any homology with the other known honeybee peptides such as royalisin, apideacin, hymenoptacin and abaecin, or with the honeybee venom peptides as melittin, apamin and MCD-peptide. The proliferation of human monocytes is regulated by Apisimin. It is demonstrated that the presence of apisimin and arabinogalactan AMPs contributing to immune active quality of honey[11] (Figs 1 & 2, and Tables 1-4).

Apalbumins (major royal jelly proteins)

The vital and reliable honeybee proteins in honey are major royal jelly proteins labelled as apalbumins (Apa). They are members of a protein family with a molecular weight ranged between 49 and 87 kDa. Approximately ninety per cent of the royal jelly protein contents include Apa 1, Apa 2 and Apa 3. Apalbumins (1-3) presenting sequence homology to the tune of 72 % and minor royal jelly proteins are predominantly the homologues of apalbumins, antimicrobial peptides, and enzymes. It is one of the most abundant acidic peptides in royal jelly and existing in royal jelly as monomer (55 kDa) and oligomer (420 kDa). Apisimin and apalbumin form an oligomeric stable complex to form gel. This peptide is

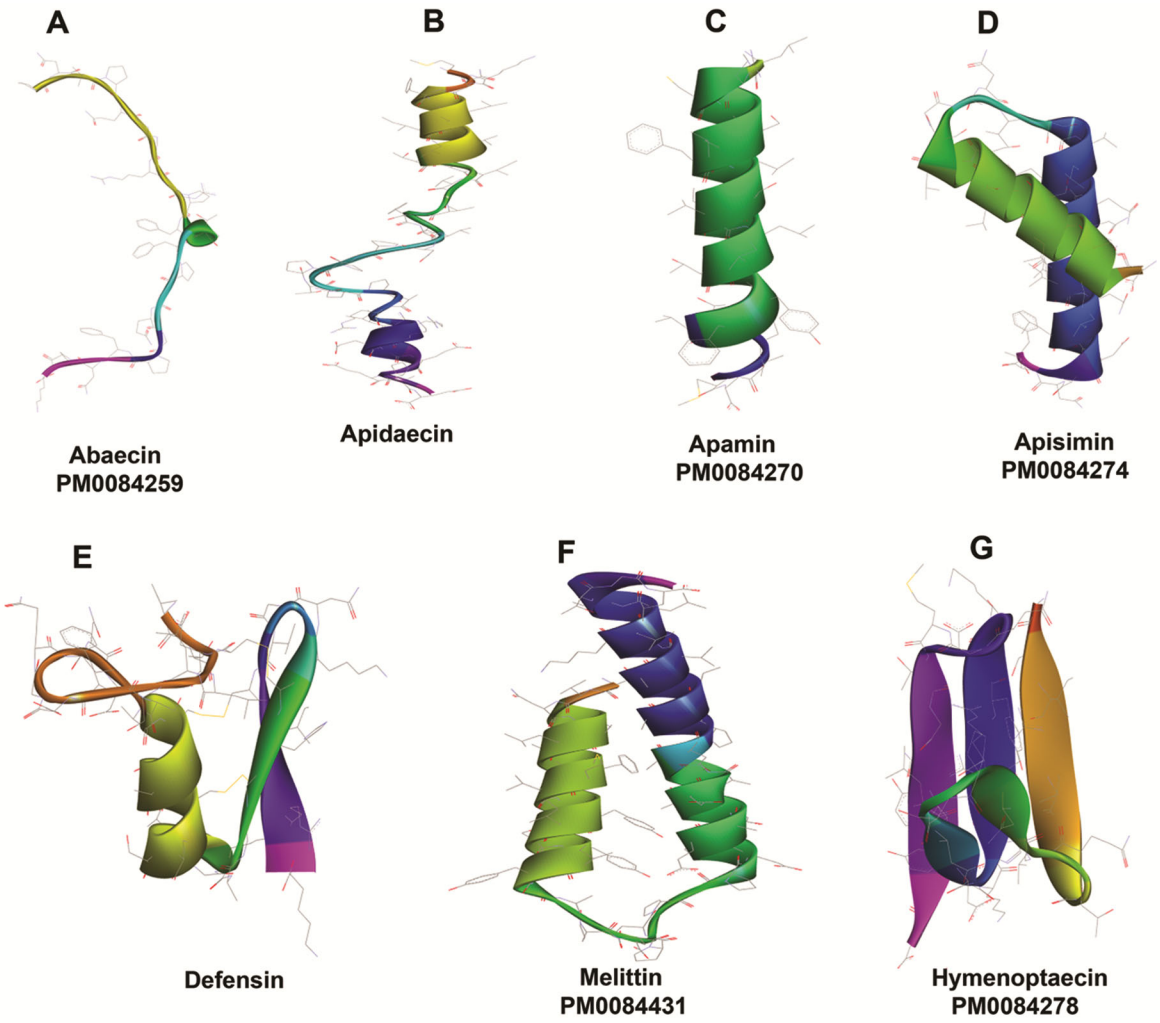

Fig. 2 — Three dimensional structure of antimicrobial peptides structured via homology modeling (SWISS model) and validated the structure by Ramachandran plot using SAVES server tool.

generated throughout the entire life period of nurse and foraging honeybees and involved in many cellular processes and cell proliferation activation alone and in complex with apisimin. Apalbumin has multifunctional roles as apa1 and apa2 induce the release of TNF-α and apa3 regulates immune response[13] (Figs 1 & 2, and Tables 1-4).

Apalbumina/Apalbumin 1

The major unique and authentic components of honey bee proteins in honey are enzymes associated with carbohydrate metabolism and royal jelly proteins. Apalbumina or apalbumin 1 is one of the most prevailing royal jelly proteins of honey. It is a versatile and multifaceted glycoprotein stimulating the TNF-α cytokine via murine macrophages as well as augmenting the hepatocytes proliferation. C-terminal portion of apalbumina is a precursor form of jelleines (I-IV). In royal jelly, apalbumina exists as a monomer (55 kDa) or in oligomeric form (420 kDa). It is actively participating (self-assembled membranes) in improving moisture content during processing of nectar to ripen honey (18% moisture content) in contrast forming filamentous spider web-like networks upon immobilization of pollen grains as pollen pellet. The ratio of apalbumin 1/apalbumina to total proteins of honey can be measured as supportive benchmark for adulteration of honey. The quantity of apa1 in honey is < 50 µg/g would be suggestive of the presence of contamination. Hence, apalbumina can be used as a quality marker to validate honey and honey bee products[11]. In addition, the apalbumina demonstrates the antimicrobial properties toward *B. subtilis* and *E. coli* (Figs 1 & 2, and Tables 1-4).

Table 3 — Prediction of antimicrobial activity and toxicity of honey bee antimicrobial peptides using CAMP database and ToxIBTL server tools

Sl. No.	Peptide name	Uniprot ID[†]	Antimicrobial activity prediction[#]				Toxicity prediction[$]	
			SVM score	RF score	ANN prediction	DA score	Prediction	Score
			Abaecin					
1	*Apis mellifera*	P15450	0.928	0.972	AMP	0.904	non-toxic	3.963
2	*Apis cerana cerana*	A0A2A3E7A0	0.911	0.947	AMP	0.901	non-toxic	5.668
3	*Apis mellifera*	D2YZ38	0.691	0.692	AMP	0.504	non-toxic	2.919
4	*Apis cerana japonica*	Q8WSY9	0.763	0.787	AMP	0.620	non-toxic	2.312
5	*Melipona quadrifasciata*	A0A0N0BGD9	0.971	0.732	AMP	0.807	non-toxic	1.788
			Apamin					
6	*Apis mellifera*	P01500	0.909	0.921	AMP	0.907	non-toxic	1.345
7	*Apis cerana cerana*	Q86QT2	0.811	0.879	AMP	0.618	non-toxic	1.410
8	*Apis mellifera*	A0A2A3EK62	1.000	0.913	AMP	1.00	non-toxic	1.295
			Melittin					
9	*Apis mellifera*	P01501	0.917	0.917	AMP	0.718	non-toxic	2.295
10	*Apis cerana*	Q8LW54	0.814	0.951	AMP	0.820	non-toxic	6.445
11	*Apis florea*	P0DPR9	0.994	0.999	AMP	0.999	non-toxic	2.625
12	*Apis dorsata*	P01504	0.977	0.997	AMP	0.992	non-toxic	1.479
13	*Apis cerana cerana*	P68407	0.825	0.833	AMP	0.651	non-toxic	1.678
14	*Apis mellifera carnica*	I3RJI9	0.917	0.917	AMP	0.718	non-toxic	2.569
Major royal jelly protein 1 and Jellein								
15	*Apis mellifera*	O18330	1.000	0.959	AMP	1.000	non-toxic	8.965
16	*Apis mellifera*	P84759	0.902	0.930	AMP	0.869	non-toxic	2.225
			Apisimin					
17	*Apis mellifera*	Q8ISL8	0.716	0.921	AMP	0.885	non-toxic	7.926
18	*Apis cerana cerana*	A0A2A3EKN9	0.902	0.994	AMP	0.863	non-toxic	2.004
Apidaecins								
19	*Apis mellifera*	Q06602	1.000	0.871	AMP	1.000	non-toxic	5.426
20	*Apis cerana cerana*	B9UKB7	1.000	0.886	AMP	1.000	non-toxic	1.150
21	*Melipona quadrifasciata*	A0A0N0U6Q6	1.000	0.941	AMP	1.000	non-toxic	2.007
			Hymenoptaecin					
22	*Apis mellifera*	Q10416	1.000	0.986	AMP	1.000	non-toxic	3.412
23	*Apis cerana cerana*	B9UKG2	1.000	0.987	AMP	1.000	non-toxic	2.051
24	*Apis dorsata*	C7AHW4	1.000	0.989	AMP	1.000	non-toxic	6.332
25	*Apis andreniformis*	C7AHW3	1.000	0.913	AMP	1.000	non-toxic	1.714
26	*Apis cerana*	V9IC30	1.000	0.913	AMP	1.00	non-toxic	1.399
			Defensin					
27	*Apis mellifera carnica*	Q5J8R1	0.997	0.979	AMP	0.994	non-toxic	1.200
28	*Apis cerana cerana*	A7L3U8	1.000	0.977	AMP	0.999	non-toxic	1.998
29	*Apis cerana*	C7AHS9	0.922	0.958	AMP	0.716	non-toxic	2.354
30	*Apis mellifera*	A0A088A8D5	0.997	0.989	AMP	0.982	non-toxic	5.833
31	*Apis dorsata*	A0A387IGF2	0.750	0.730	AMP	0.641	non-toxic	1.002
32	*Apis cerana japonica*	D3KYH2	0.747	0.806	AMP	0.929	non-toxic	2.720
33	*Melipona quadrifasciata*	A0A0N0U626	1.000	0.979	AMP	0.999	non-toxic	1.999

[†]Antimicrobial peptide sequences were retrieved from Uniprot database (https://www.uniprot.org/help/uniprotkb)

[#]Antimicrobial activity prediction of antimicrobial peptides was predicted through the CAMP database (http://www.camp3.bicnirrh.res.in) using the support vector machine (SVM), random forest (RF), artifcial neural network (ANN) and discriminant analysis (DA) algorithms for scoring. The threshold score to be considered as antimicrobial peptide is 0.5.

[$]Toxicity prediction was done using online ToxIBTL server (https://server.wei-group.net/ToxIBTL). The score >1 is considered as non-toxic and ≤1 is considered as toxic.

Apisin

Apisin is a hetero-oligomer comprising of major royal jelly protein 1 and apisimin. It is a unique protein that constitutes the larger portion of the royal jelly proteins. Apisin is known as 350 kDa major royal jelly glycoprotein-1 multimer (MRJP-1 multimer). It is containing an N-glycan with a distinctive high-mannose type oligosaccharide (Man9–5GlcNAc2). A

Table 4 — Details of antiviral, antifungal and anticancer activity prediction results of antimicrobial peptide from honey bee species using AVPpred, Antifp and iACP tools

A Sl. No	Peptide name	Uniprot ID	Antiviral activity prediction (AVP) #					Antifingal activity$		Anticancer*		
			AVP motif	Alignment model	Composition model (%)	Physio-chemical model (%)	Overall prediction	Score	Prediction	Prediction	Anticancer score	NAC score
Abaecin												
1	*Apis mellifera*	P15450	-	Non-AVP	51.23	64.08	Y	0.559	Antifungal	Anticancer	0.959	0.040
2	*Apis cerana cerana*	A0A2A3E7A0	-	Non-AVP	49.25	64.09	N	-0.295	NAF	Anticancer	0.959	0.040
3	*Apis mellifera*	D2YZ38	-	Non-AVP	49.76	64.08	N	0.067	Antifungal	Anticancer	0.9593	0.040
4	*Apis cerana japonica*	Q8WSY9	-	Non-AVP	49.99	64.08	N	-0.295	NAF	Anticancer	0.959	0.040
5	*Melipona quadrifasciata*	A0A0N0BGD9		Non-AVP	61.21	64.14	Y	0.552	Antifungal	Anticancer	0.959	0.040
Apamin												
6	*Apis mellifera*	P01500	-	Non-AVP	59.57	64.14	Y	0.535	Antifungal	Anticancer	0.959	0.040
7	*Apis cerana cerana*	Q86QT2	-	Non-AVP	61.21	64.14	Y	0.595	Antifungal	Anticancer	0.959	0.040
8	*Apis mellifera*	A0A2A3EK62	-	Non-AVP	59.24	64.14	Y	0.571	Antifungal	Anticancer	0.959	0.040
Melittin												
9	*Apis mellifera*	P01501	-	Non-AVP	47.75	64.08	N	-0.674	NAF	Anticancer	0.609	0.390
10	*Apis cerana*	Q8LW54	-	Non-AVP	59.54	64.08	Y	0.582	Antifungal	Anticancer	0.952	0.047
11	*Apis florea*	P0DPR9	-	Non-AVP	47.08	69.15	N	-0.069	NAF	Anticancer	0.959	0.040
12	*Apis dorsata*	P01504	-	Non-AVP	57.50	66.21	Y	0.528	Antifungal	Anticancer	0.959	0.040
13	*Apis cerana cerana*	P68407	-	Non-AVP	53.82	64.08	Y	0.595	Antifungal	Anticancer	0.609	0.390
14	*Apis mellifera carnica*	I3RJI9	-	Non-AVP	47.75	64.08	N	-0.674	NAF	Anticancer	0.609	0.390
Major royal jelly protein												
15	*Apis mellifera*	O18330	-	Non-AVP	35.64	64.08	N	-0.028	NAF	NAC	0.233	0.766
Jellein												
16	*Apis mellifera*	P84759	-	Non-AVP	45.18	42.90	N	-0.264	NAF	Anticancer	0.959	0.040
Apisimin												
17	*Apis mellifera*	Q8ISL8	-	Non-AVP	45.58	64.08	Y	0.528	Antifungal	NAC	0.157	0.842
18	*Apis cerana cerana*	A0A2A3EKN9	-	Non-AVP	42.79	64.08	N	-1.3901	NAF	NAC	0.038	0.961
Apidaecin												
19	*Apis mellifera*	Q06602	-	Non-AVP	28.35	64.08	N	-1.065	NAF	NAC	0.009	0.990
20	*Apis cerana cerana*	B9UKB7	-	Non-AVP	47.28	64.05	N	-0.186	NAF	Anticancer	0.551	0.448
21	*Melipona quadrifasciata*	A0A0N0U6Q6	-	Non-AVP	43.42	64.08	N	-0.028	NAF	NAC	0.354	0.645
Hymenoptaecin												

(*contd.*)

Table 4 — Details of antiviral, antifungal and anticancer activity prediction results of antimicrobial peptide from honey bee species using AVPpred, Antifp and iACP tools (*contd.*)

A

Sl. No	Peptide name	Uniprot ID	Antiviral activity prediction (AVP) [#]					Antifingal activity[$]		Anticancer[*]		
			AVP motif	Alignment model	Composition model (%)	Physio-chemical model (%)	Overall prediction	Score	Prediction	Prediction	Anticancer score	NAC score
22	*Apis mellifera*	Q10416	-	Non-AVP	35.69	64.03	N	-0.341	NAF	NAC	0.030	0.969
23	*Apis cerana cerana*	B9UKG2	-	Non-AVP	43.13	64.04	Y	0.591	Antifungal	Anticancer	0.809	0.190
24	*Apis dorsata*	C7AHW4	-	Non-AVP	32.54	64.02	N	-0.019	NAF	Anticancer	0.539	0.460
25	*Apis andreniformis*	C7AHW3	-	Non-AVP	28.64	64.04	N	-0.236	NAF	Anticancer	0.954	0.045
26	*Apis cerana*	V9IC30	-	Non-AVP	47.28	64.05	N	-0.486	NAF	Anticancer	0.614	0.386
Defensin												
27	*Apis mellifera carnica*	Q5J8R1	-	Non-AVP	44.24	64.08	N	-0.019	NAF	Anticancer	0.569	0.430
28	*Apis cerana cerana*	A7L3U8	-	Non-AVP	39.03	64.08	N	0.183	Antifungal	Anticancer	0.704	0.295
29	*Apis cerana*	C7AHS9	-	Non-AVP	41.45	64.08	Y	0.536	Antifungal	NAC	0.378	0.621
30	*Apis mellifera*	A0A088A8D5	-	Non-AVP	43.49	64.07	Y	0.507	Antifungal	NAC	0.108	0.891
31	*Apis dorsata*	A0A387IGF2	-	Non-AVP	46.06	64.07	Y	0.536	Antifungal	Anticancer	0.689	0.310
32	*Apis cerana japonica*	D3KYH2	-	Non-AVP	24.64	64.05	N	-0.295	NAF	NAC	0.085	0.914
33	*Melipona quadrifasciata*	A0A0N0U626		Non-AVP	43.13	64.04	Y	0.591	Antifungal	Anticancer	0.809	0.190

[#]Antiviral activity prediction was performed by using the online server AVPpred (http://crdd.osdd.net/servers/avppred/). It exploits four diferent models: (1) the AVP motif, which returns the result as YES or NO; (2) the Alignment model, which gives the result in the form AVP or Non-AVP; (3) the Composition model and the (4) the Physico-chemical model, which return their results in a numerical form (percentage). The overall result is expressed with a YES, if the peptide results have a putative antiviral activity, and with a NO, if otherwise.

[$]Antifungal activity prediction was performed using the Antifp server (https://webs.iiitd.edu.in/raghava/antifp/disp.php), which gives the result as a numerical score, ≥0.5 score is considered as threshold.

[*]Anticancer activity prediction was performed by iACP tool (http://lin-group.cn/server/iACP), providing the results in a numerical form. ≥ 0.5 score considered as anticancer.

Abbreviations: NAC – Non-anticancer; NAF – Non-antifungal; Y – Yes; N – No;

B

Sl. No.	Peptide name	Uniprot ID	#Antimicrobial activity prediction				$Toxicity prediction	
			SVM	RF	ANN	DA	Result	Score
Abaecin								
1	*Apis mellifera*	P15450	0.928	0.972	AMP	0.904	non-toxic	3.963
2	*Apis cerana cerana*	A0A2A3E7A0	0.911	0.947	AMP	0.901	non-toxic	5.668
3	*Apis mellifera*	D2YZ38	0.291	0.692	NAMP	0.004	non-toxic	2.919
4	*Apis cerana japonica*	Q8WSY9	0.263	0.487	NAMP	0.020	non-toxic	2.312
5	*Melipona quadrifasciata*	A0A0N0BGD9	0.971	0.432	AMP	0.807	non-toxic	0.000
Apamin								
6	*Apis mellifera*	P01500	0.909	0.921	AMP	0.907	toxic	0.999
7	*Apis cerana cerana*	Q86QT2	0.211	0.279	NAMP	0.018	toxic	0.999
8	*Apis mellifera*	A0A2A3EK62	1.000	0.913	AMP	1.00	toxic	0.999
Melittin								
9	*Apis mellifera*	P01501	0.017	0.017	NAMP	0.018	non-toxic	2.295

(*contd.*)

Table 4 — Details of antiviral, antifungal and anticancer activity prediction results of antimicrobial peptide from honey bee species using AVPpred, Antifp and iACP tools (*contd.*)

10	*Apis cerana*	Q8LW54	0.114	0.351	NAMP	0.120	non-toxic	6.445
11	*Apis florea*	P0DPR9	0.994	0.999	AMP	0.999	non-toxic	2.625
12	*Apis dorsata*	P01504	0.977	0.997	AMP	0.992	toxic	0.999
13	*Apis cerana cerana*	P68407	0.025	0.033	NAMP	0.051	toxic	0.999
14	*Apis mellifera carnica*	I3RJI9	0.017	0.017	NAMP	0.018	non-toxic	2.569
Major royal jelly protein 1 and Jellein								
15	*Apis mellifera*	O18330	1.000	0.959	NAMP	1.000	non-toxic	8.965
16	*Apis mellifera*	P84759	0.102	0.330	AMP	0.169		
Apisimin								
17	*Apis mellifera*	Q8ISL8	0.516	0.321	NAMP	0.885		
18	*Apis cerana cerana*	Q8ISL8	0.602	0.394	AMP	0.863	non-toxic	2.004
Apidaecins								
19	*Apis mellifera*	Q06602	1.000	0.871	AMP	1.000	non-toxic	5.426
20	*Apis cerana cerana*	B9UKB7	1.000	0.886	NAMP	1.000	non-toxic	1.150
21	*Melipona quadrifasciata*	A0A0N0U6Q6						
Hymenoptaecin								
22	*Apis mellifera*	Q10416	1.000	0.986	AMP	1.000	non-toxic	3.412
23	*Apis cerana cerana*	B9UKG2	1.000	0.987	AMP	1.000	toxic	0.999
24	*Apis dorsata*	C7AHW4	1.000	0.989	AMP	1.000	non-toxic	6.332
25	*Apis andreniformis*	C7AHW3	1.000	0.913	AMP	1.00	toxic	0.999
26	*Apis cerana*	V9IC30	1.000	0.913	AMP	1.00	toxic	0.999
Defensin								
27	*Apis mellifera carnica*	Q5J8R1	0.997	0.979	AMP	0.994	toxic	1.000
28	*Apis cerana cerana*	A7L3U8	1	0.977	AMP	0.999	toxic	0.998
29	*Apis cerana*	C7AHS9	0.922	0.658	AMP	0.516	non-toxic	2.354
30	*Apis mellifera*	A0A088A8D5	0.997	0.989	NAMP	0.982	non-toxic	5.833
31	*Apis dorsata*	A0A387IGF2	0.750	0.430	AMP	0.641	non-toxic	1.002
32	*Apis cerana japonica*	D3KYH2	0.747	0.406	AMP	0.229	non-toxic	2.720
33	*Melipona quadrifasciata*	A0A0N0U626	1.000	0.979	AMP	0.999	toxic	0.999

#Antimicrobial activity prediction was predicted through the CAMP database. CAMP gives four score *i.e.* (i) Support Vector Machine (SVM) score, (ii) Random Forest (RF) score, (iii) Artifcial Neural Network (ANN) result and (iv) Discriminant Analysis (DA) score. The threshold score to be considered as antimicrobial peptide is 0.5; $Toxicity prediction was done by using ToxIBTL server. The score >1 is considered as non-toxic and ≤1 is considered as toxic

galactosyl residue in N-glycans is a natural bee glycoproteins linked to royal jelly glycoprotein mixture. Apisin content was estimated to be more than 1,500 mAU in natural royal jelly samples from Japan. Japan royal jelly INC conducted a study and found that bee larvae fed with royal jelly containing higher content of apisin (1500 mAU) grew to a size several times greater at 24 hours after feeding, and grew incredibly fast into queen bees. These observations evidenced that the growth of bee larvae is affected by apisin and is a key substance in the growth of larvae into queen bees[14] (Figs 1 & 2, and Tables 1-4).

Royalisin

This is a member of the insect defensin family (5.5 kDa, six cysteine/three disulfide bridge pattern) derived from the royal jelly of *A. mellifera*. It consists of 51 amino acids, a characteristic disulfide-rich structure (40 amino acids), a distinct amphipathic α-helix, an amidated carboxyl-terminal tail (11 amino acids) and having compact globular structure because of forming three intramolecular disulfide linkages via six cysteine residues[15]. This bee AMP demonstrates widespread sequence homology to antibacterial proteins as sapecin and phormicins. The presence of intramolecular disulfide linkages and the 11 amino acids at the C-terminus of royalisin ensures their higher antimicrobial against gram-positive bacteria (*Clostridium*, *Corynebacterium*, *Leuconostoc*, *Staphylococcus*, *Bacillus subtilis*, *Sarcina lutea* and *Streptococcus*), gram-negative bacteria, American foulbrood disease (*Paenibacillus larvae*) and fungi (*Botrytis cinerea* and *Alternaria brassica*) than the royalisin-D (shortened royalisin)[15]. Royalisin (180 μg/mL) inhibits *B. subtilis* significantly in comparison with tetracyclin (50 μg/mL). Royalisin can be used as a food preservative and a potent drug against diseases because of its stable nature at low pH and high temperature (Figs 1 & 2, and Tables 1-4).

Jelleines

The jelleines are hydrophobic, cationic antimicrobial peptides shorter than the most AMPs. They are containing 8 to 9 amino acid residues with a net charge of 1+ or 2+ while the other AMPs having 12 to 50 amino acids with a net positive charge between 2+ and 7+. Four forms of jelleines were isolated from royal jelly of honeybees, *i.e*., Jelleine-I (PFKLSLHL-NH2), Jelleine-II (TPFKLSLHL-NH2), Jelleine-III (EPFKLSLHL-NH2), and Jelleine-IV (TPFKLSLH-NH2). Jelleines encompass surplus of basic amino acid residues and hydrophobic residues (> 50%) which are having direct interactions with bacterial membranes. Jelleine-I, is an amphiphilic AMP (953.17 kDa) showing potent *in vitro*, *in vivo* antimicrobial (neutralize lipopolysaccharide, interrupt the integrity of bacterial cell membrane, intermingle with bacterial genomic DNA and cause the release of ATP) and antifungal activity[16]. The Jelleines (I–III) are showing antimicrobial and anti-fungal activities against Gram-positive bacteria (*Styphylococcus aureus*, *S. saprophyticus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). No cytolytic activity and induction of rat peritoneal mast cell degranulation were observed in the Jelleines (I–IV). The jelleine-IV lacks antibacterial, antifungal, cytolytic activity as well as stimulation of rat peritoneal mast cell degranulation[16] (Figs 1 & 2, and Tables 1-4).

10 HDA and 10 H2DA

10-hydroxy-2-decenoic acid (10HDA, queen bee acid) and 10-Hydroxy-trans-2-decenoic acid (10H2DA, royal jelly acid) are the distinctive major lipid components of royal jelly and present only in royal jelly. 10H2DA (> 100 mM concentration) presents only in royal jelly comprising 50% of the total fatty acid content followed by 10HDA (1.5 – 2.0%) which is a saturated lipid form of 10H2DA. Out of 3-8% lipids of royal jelly, more than 60-80% contains both 10H2DA and 10HDA. Hence, these host defense peptides could be employed as biomarkers as well as internal standards to authenticate quality of royal jelly and straightaway decides the value of royal jelly on the international market. 10HDA and 10H2DA have been reported to have biological activities as immunomodulatory, anti-bacterial (*E. coli*, *B. subtilis*, *Micrococcus pyogenes* and *Staphylococcus aureus*), anti-fungal (*Neurospora sitophila*), anti-rheumatoid arthritis, anti-cancer, anti-angiogenesis, promotion of collagen production, T-lymphocytes and IL-2, epigenetic regulation, modulation of ion channels, estrogenic and neurogenic modulation[16] (Figs 1 & 2, and Tables 1-4).

Hymenoptaecin

The *A. mellifera* hymenoptaecin is a long (93 amino acids) glycine rich cationic AMP, including a 2-pyrrolidone-5-caboxylic acid at the N-terminus. The precursor of bee hymenoptaecins comprises a signal peptide, pro-sequence and a mature peptide. The propeptide of bee hymenoptaecins is showing homology with the wasp *Nasonia vitripennis* and encoding multipeptide precursors. In *A. cerana* adult workers, thirteen different hymenoptaecin peptides were identified. In the wild *A. mellifera* exposed to mild pathogen infection, only the genes for apidaecin and hymenoptaecin were expressed, whereas abaecin and defensin were absent[11] (Figs 1 & 2, and Tables 1-4).

Defensin

It is a family of cystein-rich cationic AMPs (36-51 amino acids) consisting of an α-helix, two antiparallel β-strands and a loop at the amino end which are stabilized by three disulfide bridges. Defensin is the prime defense system in bees and consists of two types namely defensin 1 (5.5 KDa) and 2 (4.8 KDa) modulated by Toll and Imd signal pathways. Defensin 1 is produced in salivary glands and also exists in the royal jelly and honey associated with social immunity while defensin 2 is synthesized in fat body and haemolymph of bee linked with individual immunity. *A. mellifera* defensin AMP comprises 43 amino acids (VTCDVLSWQSKWLSINHSACAIRCLAQRRKGG SCRNGVCICRK), whereas other bee species contains 51 amino acids because of additional amino acid replacements amongst bee defensin peptides. The open reading frame of *A. cerana* defensin contains an additional amidated amino acid, glycine at the carboxy terminal compared with *A. mellifera* defensin. The structure of defensin is showing similarity with *Phormia terranovae* and homology between the defensin 1 and 2 is found to be 55.8%[18]. Three isoforms of Defensin 1 were isolated in honey bee, *i.e*., 1 isoform from hemolymph and 2 isoforms from royal jelly [royalisin – Ro-F (5.525 KDa) and Ro-K (5.515 KDa,)]. The haemolymph isoform 1 defensin variant is varied from the royalysin defensin variants by two amino acids substitutions. The genes

defensin 1 and 2 differ significantly by the length (2012 and 1950 bp), intron-exon structure [2 introns (between 773-1345 and 1525-180 and 1 intron (between 947 and 1283)], pre-pro-peptide region (defensin 2 longer than defensin 1) and mature peptide (51 and 43 amino acids and absence of C-terminal elongation in defensin 1), respectively[18]. proved the close phylogenetic relationship (93%) between *Apis cerana japonica* defensin (AcjDef2) and *Apis mellifera* defensin (AmDef). Defensin showing selective antipathogenic activity against *Paenibacillus larvae*, *Melissococcus pluton*, *Ascosphaera apis*, *Nosema apis*, *N. ceranae*, *Aspergillus flavus*, *A. niger*, *Candida albicans*, *Aurobasidium pullulans*, *Varroa destructor*, *V. jacabsoni*, *Acarapis woodi*, and *Tropilaelaps clareae*[18]. Modulations in antipathogenic activity of the honeybee defensins are found to be observed because of alterations in processing of defensin precursors that lead to generation of molecular variants (29 varied defensin cDNA genes coding 7 diverse defensin peptides)[18] (Figs 1 & 2, and Tables 1-4).

Apamin

Apamin (H-Cys-Asn-Cys-Lys-Ala-Pro-Glu-Thr-Ala-Leu-Cys-Ala-Arg-Arg-Cys-Gln-Gln-His-NH_2) is one of the AMPs found in bee venom (2-3%). It is a globular neurotoxin peptide having 18 amino acid residues and compactly networked with two disulfide bonds. It is an inflexible polypeptide due to the presence of two disulfide bridges between Cys_1- Cys_{11} and Cys_3 - Cys_{15} position and seven hydrogen bonds[19]. It possesses many pharmacological activities as blocking calcium-activated potassium channels, regulation of cell growth via signal transduction pathways, alleviating neurodegeneration, neurofibromatosis, acute pancreatitis, liver fibrosis, atopic dermatitis, atherosclerosis, rheumatoid arthritis, multiple sclerosis, cognitive deficit and dementia and inhibition of Th2-related cytokines, TNF-α- and IFN-γ[19]. (Figs 1 & 2, and Tables 1-4).

Melittin

It is a main AMP of bee venom (40-60%) contains 26 amphipathic amino acids (**H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2**), no disulfide bridge, 6 positive charges located in C-terminal responsible for α-helix stability and no negative charges. It is a small polypeptide denatured in aqueous solutions and folding spontaneously in non-polar media[20]. The amino-terminal portion of melittin is principally hydrophobic with lack of lytic activity whereas the carboxyl-terminal region is hydrophilic, strongly basic and accountable for the lytic action. Melittin (monomeric and tetrameric forms) is soluble in water because of its amphipathic nature. The mode of action of melittin is disrupting the prokaryotic and eukaryotic cell membranes through pore formation and lysis. It is forming transient and stable pore formation when cell membrane orients and permeable to ions and large molecules (glucose), respectively. Melittin is being involved in diverse biological activities as the release of pro-inflammatory cytokines, activates G-protein-coupled receptor-mediated opening of transient receptor potential channels, inhibits protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II, myosin light chain kinase, Na+/K+-ATPase (synaptosomal membrane) and blocks transport pumps (Na^+-K^+-ATPase and the H^+-K^+-ATPase), anti-nociceptive and haemolytic, activator of PLA2, anticancer, antitumor, antidiabetic, anti-biofilm, anti-inflammatory, antibacterial, antifungal, antiviral, anti-arthritic and the immune response of bees to infectious diseases[21,22] (Figs 1 & 2, and Tables 1-4).

Melectin

This AMP contains 18 amino acids, cationic (net charge, +5), amphipathic and α-helical structure with a molecular weight of 2038.3 kDa. It has hydrophobic and basic amino acid residues and a proline (H-**GFLSILKKVLPKVMAHMK-NH_2**). Despite short amino acid sequence, low cytotoxicity in mammalian cells, broad-spectrum potency, cell selectivity, salt-resistant properties and rapid bactericidal action than melittin (26 amino acids) melectin exhibits significant antimicrobial action against gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *E. coli*) including drug-resistant bacteria (*S. aureus* MRSA 254/366/660, *P. aeruginosa* 1034/3543/5018 and *E. coli* CCARM 1229/1238). Melectin possesses the biological activities as selectivity of bacterial cells via pro kink in the α-helical structure, degranulation of peritoneal mast cells in rats, low haemolytic activity and anti-tumor[11] (Figs 1 & 2, and Tables 1-4).

Adolapin

Adolapin is one of the components the honey bee venom and constitutes about 0.1-1% in dry venom[19]. It contains 2-5% of the AMPs in bee venom and a

basic AMP (11.5 kDa) comprises 103 amino acids and is showing marked pharmacological properties such as anti-inflammatory, anti-nociceptive, analgesic, antipyretic, inhibition of COX1, prostaglandin synthase and lipoxygenase systems in human platelets, and upsurge c-GMP level in the rat spleen and brain and declines the c-AMP level in the rat spleen[19] (Figs 1 & 2, and Tables 1-4).

Mast Cell Degranulating (MCD) Peptide

MCD or peptide 401 (2587.2 kDa) is one of the cationic (net charge +8), bicyclic peptide components of bee venom (1-3% of dry venom)[21]. It is composed of 22 amino acids (H-**IKCNCKRHVIKPHIC RKICGKN-NH_2**) with two disulfide associations between Cys_3 - Cys_{15} and Cys_5 - Cys_{19}. MCD secondary structure is analogous with apamin AMP. It has several biological functions as immunological and pharmacological activities as analgesic and nociceptive effects and associated with allergy, releasing low concentrations of histamine, potent natural histamine secretagogues, inhibiting mast cell degranulation, anti-allergic agent, potent blocker of voltage-sensitive potassium channels, anti-inflammatory and can be used as a vaccine adjuvant. MCD acts as a mediator at low concentrations inducing mast cell degranulation and the release of histamine, resulting in inflammation processes while at high concentration, MCD inhibits mast cell degranulation and therefore, exerts anti-inflammatory effects[23]. It is acting as an epileptogenic neurotoxin, and an inhibitor of potassium channels instigating dropping of blood pressure in rats. At high concentration, it acts as an anti-allergen because of the presence of disulfide linkage which shares between IgE and the MCD peptide on the mast cell receptors to inhibit mast cell degranulation and to prevent the discharge of histamine and consequently, put forth anti-inflammatory effects[23] (Figs 1 & 2, and Tables 1-4).

Secapin

It is a non-toxic polypeptide of bee venom (0.5-2% dry venom) composed of 25 amino acids (YIIDVPPRCPPGSKFIKNRCRVIVP-NH_2) and rich in proline with a disulfide bond between the Cys_9 and Cys_{20} residues[19]. So far, many secapin isoforms [secapin, secapin-1, secapin-2 and secapin-3) were isolated from *A. mellifera* as well as AcSecapin-1 from *A. cerana*[19]. The amino acid sequences of three isoforms of this AMP are as follows: secapin (YIIDVPPRCPPGSKFIKNRCRVIVP-NH_2), secapin-1 (YIINVPPRCPPGSKFVKNKCRVIVP-NH2) and secapin-2 (YIIDVPPRCPPGSKFV HKRCRVIVP-NH2). The differences between secapin-1 and secapin-2 isoforms were substitutions at amino acids 17-19. AcSecapin-1 showed higher sequence similarity with *A. dorsata* secapin, *A. florea* secapin and *A. mellifera* secapin-3 than AcSecapin. AcSecapin-1 acts as a serine protease-like peptide, anti-fibrinolytic, anti-elastolytic, and anti-microbial activities and exhibits inhibitory effects against trypsin, chymotrypsin, microbial serine proteases, elastases, and plasmin. Secapin-2 is not inducing hemolytic activity, mast cell degranulation, or PMNL chemotaxis; however, it influences hyperalgesia and edema mediated by leukotrienes[24] (Figs 1 & 2, and Tables 1-4).

Procamine

It is one of the AMPs in bee venom and blocks the activity of proteases as trypsin, chymotrypsin, plasmin and thrombin, as a consequence lessening inflammation[11] (Figs 1 & 2, and Tables 1-4).

Tertiapin

This AMP contains 21-amino acids, two disulfide bridges and an amidated form of carboxy terminal residue. It occupies a minor constituent of bee venom (0.1% of dry venom) and belongs to neurotoxin peptide family alike apamin. It can be used as a potassium channel modulator. In human, it blocks inward-rectifier potassium channels[11] and prevents atrioventricular transmission disorders (Figs 1 & 2, and Tables 1-4).

Cardiopep

Cardiopep (cardioactive polypeptide, 1940 kDa) includes 0.7% of bee venom and contains low molecular weight peptides to the tune of 50%. Cardiopep is less toxic (LD_{50}:15 mg/kg in mice) than phospholipase A (LD_{50}:1-5 mg/kg), melittin (LD_{50}: 2-5 mg/kg) and whole bee venom (LD_{50}: 3-5 mg/kg). Along with less toxic, it has beta-adrenergic-like stimulant and anti-arrhythmic properties[11] (Figs 1& 2 and Tables 1-4).

Bee Immune interactions

Microbial pathogens recognition by bees

Microorganisms (bacteria, fungi, viruses and parasites) are recognized differentially by the innate and cellular immune systems. The bee innate immune system recognizes PAMPs (Pathogen-Associated Molecular Patterns), which are conserved and dynamic protein structures exist in distinct pathogen

and parasite groups namely lipopolysaccharides (LPS), lipotheicoic acid, zymosan, glycolipids, glycoproteins or dsDNA. The innate immune system of bees also distinguishes DAMPs (Damage-Associated Molecular Patterns) or thermal shock protein, MAMPs (microbe-associated molecular patterns), and VAMPs (virus-associated molecular patterns), which are molecules expressed in immune cells in response to pathogenic damage. PAMPs, DAMPs, MAMPs and VAMPs act as exogenous ligands and are recognized by PRR (Pattern Recognition Receptor), PGRP (Pathogen recognition peptidoglycan recognition proteins), AMPs or peptides, which are exist in soluble form or in immune system cells[11]. PGRP-S2 and PGRP-LC are generated for the Toll and Imd pathway, respectively in response to pathogen infections. GNBP1 (Gram-negative bacteria-binding protein) recognizes 1,3 glucans and LPS present in Gram-negative bacteria, fungi and also certain Gram-positive bacteria. All the pattern recognition proteins together with serine proteases activate the splitting up of Spaetzle and Toll's endogenous ligand which are activated during immune response. In the bee genome, two orthologous genes of the Spaetzle family were identified. Recognition of microbial structures activates two main events, *i.e.*, signaling (takes place once Toll and/or IMD receptors are stimulated), and phagocytosis. The genes DSCAM and Eater are associated with endocytosis in bees. Trans-generational immune priming (transfer of immunity from mother to offspring) is facilitated by vitellogenin, egg-yolk protein in honeybees (Figs 3 & 4).

Immune response regulation in sting and stingless bees

Immune responses against pathogens and parasites include a series of proceedings that can be categorized into three phases: 1) recognition, 2) activation of signaling pathways and 3) cellular and humoral

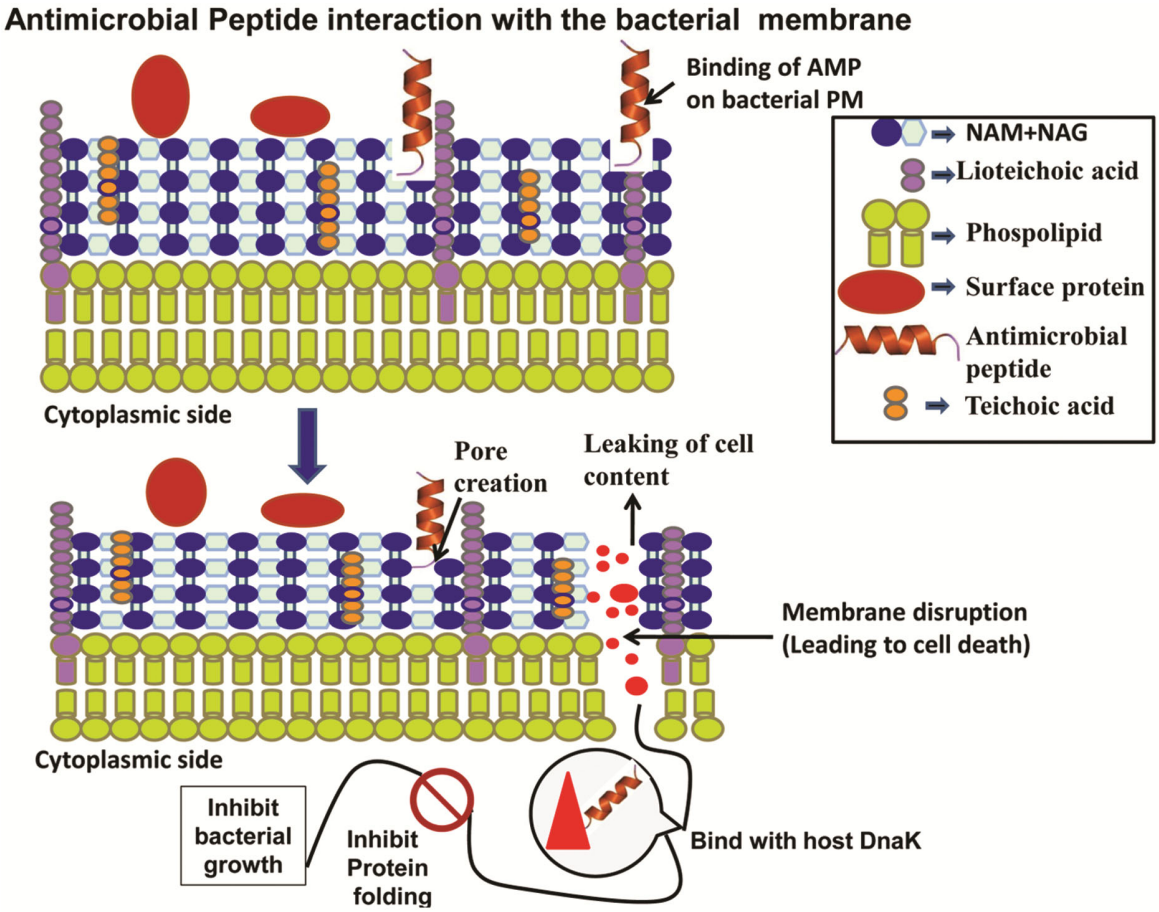

Fig. 3 — Schematic representation of antimicrobial peptides interaction with the bacterial plasma membrane and membranolytic mechanisms begin with adsorption of antimicrobial peptides on target cell membrane

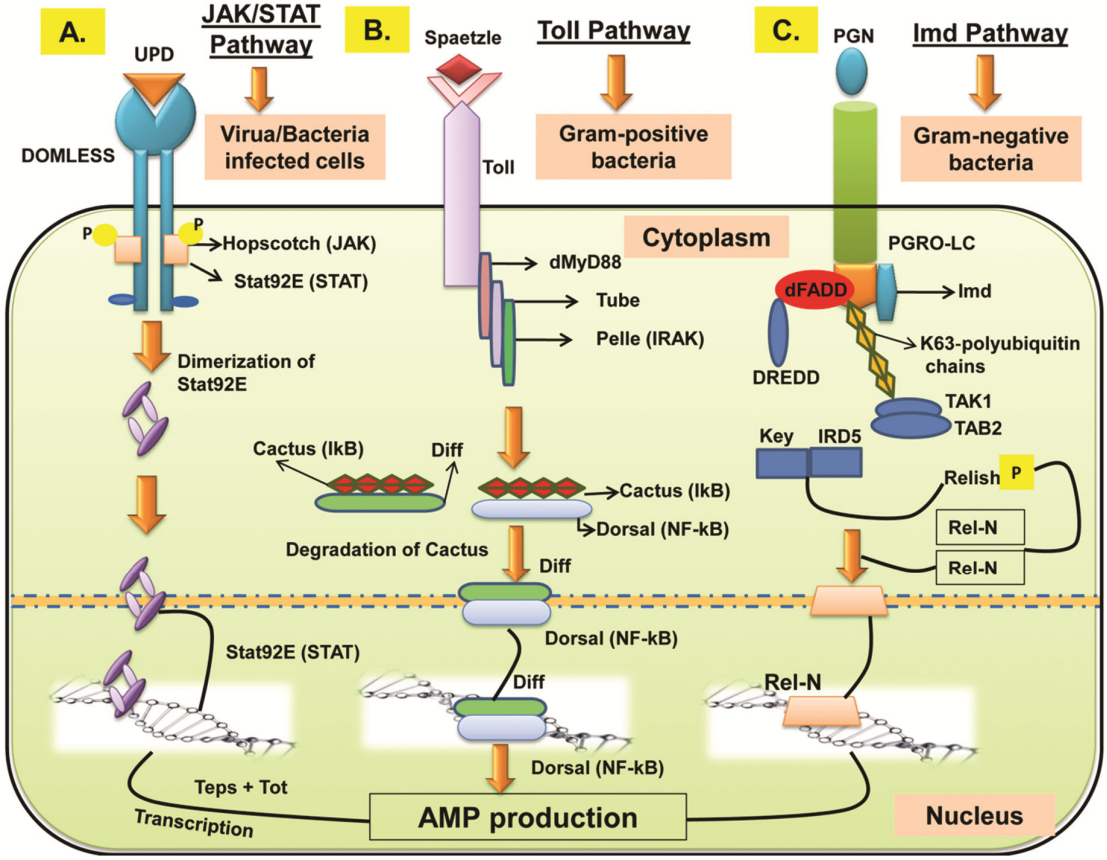

Fig. 4 — Immune pathways (JAK/STAT, Imd, and Toll) and defence mechanisms in honey bees (Manniello et al., 2021). (A) In JAK/STAT pathway, the cytokine receptor, Domeless (Dome) is activated when it binds to Unpaired (Upd) cytokines which induces the JAK tyrosine kinase Hopscotch (Hop) to phosphorylate itself, and the Dome cytoplasmic component. Consecutively, the signal transducer and activator of transcription at 92E (Stat92e) bind to the phosphotyrosines on Dome, and they are phosphorylated by Hop. Phosphorylated Stat92e separates itself from the receptor, dimerize and move into the nucleus, where it induces the transcription of Thioester-containing protein genes (Teps) and Turandot (Tot) genes for the production of AMPs; (B) Schematic representation of Toll signaling pathway. Pathogen recognition peptidoglycan recognition proteins (PGRP) activate a serine proteases cascade, involving ModSP and Grass proteins, which in turn, cleaving the inactive form of Spätzle protein, switch on the molecule. These interactions initiate protease cascades. Spätzle activates the dimer Toll receptor, which, in turn recruits cytoplasmic proteins (dMyD88, Tube, and Pelle) involved in the activation of Cactus signaling. In normal cellular condition, Cactus protein is coupled with the Nuclear Factor kappa B (NF-κB) transcription factors Dorsal-related immunity factor (DIF) and Dorsal, but following the Toll pathway activation, it is phosphorylated, detached from DIF and Dorsal and degraded. Then, both DIF and Dorsal can translocate in the nucleus and induce the transcriptional regulation of specific AMP genes; and (C) The Imd signaling pathway is activated following the binding between PGRP-LC and meso-diaminopimelic acid (DAP)-type peptidoglycan of Gram-negative and some Gram-positive bacteria. The Imd protein is activated following the cleavage by the Fas-associated death domain (FADD) and the death related ced-3/Nedd2-like caspase (DREDD). The K63-polyubiquitin chains help to link this complex with TAK1 and TAB2 proteins that, in turn, act on the IKK complex, which phosphorylates the NF-kB-like nuclear factor Relish. Consequently, TAK1 and TAB2 proteins are activated, that in turn, act on the IKK complex, composed of Immune Response Defcient 5 (IRD5) and Kenny (Key). This activated complex cleaves Relish. In this way, the Rel DNA-binding domain is released from the C-terminal ankyrin-repeat/IκB-like domain, and translocates to the nucleus inducing specific AMP genes transcription

effector mechanisms targeted at destroying pathogens and parasites. The immune response is elicited by the recognition process wherein PAMPs, DAMPs, MAMPs and VAMPs molecules resultant from pathogens and parasites. In response, different signaling pathways are triggered, validating generation of the effectors (AMPs), receptors (PRR) and PGRP involved in the humoral and cellular immune response by bees[11] (Figs 3 & 4).

Activation of AMP genes, Immune Signaling pathways, and mechanisms of action

Numerous immune signaling molecules (AMPs) are triggered in sting and stingless bees after the

immune challenge by different pathogens and parasites. The expression of AMPs are mainly regulated by the three intracellular signaling pathways by which bees showing defense against pests, parasites and pathogens, *i.e*., the Toll pathway, the Immune deficiency (Imd) pathway and the JAK-STAT pathway. Until now, there is an insufficiency and a research gap about the insights into the regulatory mechanisms of sting and stingless bees AMPs[11] (Fig. 4).

Toll pathway

In the bee immune system TLRs (Toll-like receptors) belong to a category of PRR proteins play a significant role in the innate immune system (Fig. 4). TLRs (single-pass membrane-spanning receptors) recognize all the molecular patterns (PAMPs, DAMPs, MAMPs and VAMPs) molecules of bee pathogens. In bees, the Toll pathway is predominantly for the recognition of fungi and Gram-positive bacteria by initiating cellular immune response[25]. Subsequently, humoral immune responses are triggered by the signaling pathways toward the generation of AMPs from the fat body. In response to bacterial or viral antigens, the NF-κB (nuclear factor kappa-light chain enhancer of activated B cells) is synthesized by the Toll pathway stimulus[25]. PGRPs actuate a chain of events of serine proteases along with ModSP and Grass proteins. The inactive "Spatzle protein" (extracellular cytokine like polypeptide) is cleaved and activated by ModSP and Grass proteins and in response the active Spatzle mediates to switch on the transmembrane dimer "Toll" receptor. Hence, Toll signaling pathway is initiated after Spätzle binds with the Toll receptor. The key modulator of the Toll pathway stimulus is facilitated by PGRP-SA, PGRP-SD and GNBP1 for Gram-positive bacteria while GNBP 3 for fungi. Afterward Toll-interleukin receptor domains interact with the two adaptor proteins namely MyD88 (Myeloid differentiation primary response 88) and Tube. Both these adaptor proteins mediate phosphorylation of Pelle (protein kinase) as well as phosphorylation and degradation of Cactus (IκB inhibitor). Recruitment of dMyD88, Tube, and Pelle cytoplasmic adaptor proteins by the Toll receptor is accountable for the commencement of Cactus signaling[25]. In general, Cactus protein is attached with the NF-κB (Nuclear Factor kappa B) transcription factors as DIF (Dorsal-related immunity factor) and Dorsal. Nevertheless succeeding the activation of Toll pathway and phosphorylation of Cactus, both the DIF and Dorsal are detached from the Cactus, translocated into the nucleus to synthesize AMPs[25]. As a whole activation of Toll pathway include detachment of Spaetzle, stimulation of Toll receptors, employment of cytoplasmic adapter proteins (dMyD88, Tube, and Pelle), degradation of CACTUS, activation of kinases and nuclear factors (DIF and Dorsal) and generation of AMPs (Fig. 4).

Imd pathway

Detection and recognition of pathogens in bees are mainly by way of peptide-glucan recognition protein (PGRP-LC) and this process is the primary stage for the onset of immune response through immune-deficiency (Imd) signaling pathway (Fig. 4). In bees, this path way is vital to defend against Gram-negative and some Gram-positive bacteria and modulates expression of most of the AMPs. This pathway is initiated by the assemblage between DAP-type peptidoglycan 2 (meso-diaminopimelic acid in Gram negative and some Gram positive bacteria) and PGRP-LC receptors. Initially binding of FADD (Fas-associated protein with death domain) with Imd protein facilitated the recruitment of DREDD (FADD-death-related ced-3/Nedd2-like protein) caspase like protein to split the Imd protein. After cleavage of Imd protein it is activated by K63-ubiquitination which assists Imd-K63-polyubiquitin complex to recruit TAK1 (transforming growth factor beta (TGF-β)-activated kinase 1) and TAB2 (TGF-β-activated kinase 1 (MAP3K7) Binding Protein 2) proteins. These proteins are involved with IKK complex containing IRD5 (Immune Response Deficient 5) and Key (Kenny) to enable phosphorylation of Relish (NF-kB-like nuclear factor). The triggered TAK1-TAB2 proteins and IKK-IRD5-Key complex cleave the Relish. The Rel DNA-binding domain is free for release from the IκB-like domain after cleavage and phosphorylation of Relish. Afterwards Rel DNA-binding domain is transported to the nucleus, activated and transcribed into specific AMPs. Imd signaling pathway is significantly conserved in bees (presence of orthologues – IRD5, Key, TAK1, TAB2, relish), showing high homogeneity with dipterans with different biological functions[11]. In addition, activation of components of the JNK signaling pathway (Basket, JNK and JNK-protein 1 interaction) in bees is modulated by the Imd pathway also (Fig. 4).

JAK/STAT pathway

In bees, the JAK/STAT {Janus-family tyrosinkinases [JAK]/transcription activator proteins

[STAT])} signaling pathway is mainly activated for the generation of AMP effectors against viruses, bacteria and infected cells (Fig. 4). The involvement of synthesis of AMPs by JAK/STAT pathway is analogous to the complement system, phagocytosis and antiviral feedbacks[11]. The JAK/STAT pathway is indispensable for the synthesis of cytokines in higher vertebrates and is comparatively faster. In this signaling pathway the dimerized transcription factors, STATS are initially phosphorylated for the production of AMPs by means of recruitment of the receptor ligand. JAK /STAT signaling pathway ligand is absent in bees[11]. JAK/STAT pathway in bees is comprised of five components homologous to fruit fly genes namely, a) dome (DOMELESS cytokine receptor), b) Hop (hopscotch, JAK tyrosine kinase), c) STAT92E signal transducer and transcription factor, d) SOCS (suppressors of cytokine signaling proteins – negative pathway regulators), and e) PIAS (protein inhibitor of activated STAT). In JAK-STAT pathway, the Janus kinase and STST molecule are acting as a signal transducer as well as transcription activator. In bees, JAK/STAT pathway is stimulated after binding of the Dome with unknown ligand. This Dome-ligand complex is activated the Hop and is proceeded for phosphorylation of Dome and Hop. Altogether, STAT92E is attached to the specific phosphor-tyrosine residues of Dome and later this complex is phosphorylated by Hop. After phosphorylation these complex residues are integrated with STAT molecules[11]. The integrated complex, STAT92E-STAT is then again phosphorylated by JAKs for the stimulation of immune response genes, split from the receptor to form dimers and transported to the nucleus where it is transcribed into several thioester-carrying proteins (TEPs) and tyrosine phosphatases (Ptp61F and WD40). These proteins are involved in phagocytosis and melanization processes[11] (Fig. 4).

Pharmacological properties and therapeutic potential of AMPs

Honeybees produce honey, royal jelly, propolis, bee venom, bee pollen, and beeswax, which may benefit humans due to the AMPs and other bioactives in them. Clinical standardization of these products is hindered by chemical variability depending on honeybee and botanical sources, but different molecules have been isolated and pharmacologically characterized. In apitherapy, AMPs are commonly used in the treatment of various diseases and alterations of the human being, which are present in various honey bee products, including honey, beeswax, royal jelly, propolis, bee bread, and venom (Table 1).

Honey

Defensin-1 is secreted by honeybees in the hypopharyngeal gland and is eventually transported to honey. This peptide has been identified in Revamil source honey and is responsible for its antibacterial activity against Gram-positive bacteria[26]. Apalbumin-1 isolated from Ziziphus honey inhibited ROS release (zymosan-activated human neutrophils and murine macrophages), NO production, phagocytosis (LPS-activated murine macrophages), and the production of TNF-α (human monocytic cells)[27] (Table 1).

Bee venom

Bee venom has an anti-inflammatory, antibacterial, antiviral, anti-cancer, anti-mutagenic, anti-nociceptive, and radioprotective activity[20] (Table 1). It has been used for the treatment of dermal diseases, blood circulatory system diseases, immune-related defects, tumors, back pain, rheumatism, and arthritis[29]. Bee venom contains mast cell-degranulating peptide (MCDP), melittin, adolapin, melectin, tertiapin, secarpin, minimine, procamine, and apamin[19,30] (Table 1).

Melittin

Melittin, one of the AMPs found in bee venom, has been used against prostate, liver, renal, and cervical cancers[31]. Apitoxin and its components are mostly described to have anti-inflammatory, anticancer, anti-arthritic, and neuroprotective effects[19]. Melittin is known for its anti-inflammatory properties in treating various diseases, such as dermatitis, neuritis, liver inflammation, atherosclerosis, and arthritis[32]. It has also been shown to have strong antimicrobial properties against methicillin-resistant *S. aureus*, anticancer activities, and to induce apoptosis in human ovarian cancer cells (SKOV3 and PA-1). The administration of melittin can induce the body to produce cortisol and acts as a potent anti-inflammatory representative of BV[29] (Table 1).

Apamin

Apamin has the ability to cross the blood-brain barrier, and its exposure to animals alleviates cognitive deficits, signifying that apamin targets (SK channels) would be suitable for the treatment of neurodegenerative disorders. In addition, the possibility of using apamin or less toxic analogs as

blood-brain barrier drug-delivery shuttles has been explored. It possesses anti-fungal, anti-viral, anti-inflammatory, analgesic and neurogenesis action[21,22] (Table 1).

MCD

MCD peptide consists of 22 amino acid residues having 2 disulfide bridges and possesses anti-inflammatory potential[33] (Table 1).

Adolapin

Adolapin possesses anti-arthritic, analgesic, anti-inflammatory, antipyretic, anti-nociceptive and anti-inflammatory properties[21] (Table 1).

Royal jelly

Royalisin, jellein, apisin, 10Hda, apalbumin, royalactin, and apamin are the most abundant antimicrobial peptides (AMPs) in royal jelly, which have antidiabetic, antifungal, antiviral, hypotensive, estrogen-like effects, antitumor, antiaging, antihypercholesterolemic, wound healing, and anti-inflammatory activities (Table 1). The stimulation of larval development into queen bee has been attributed to royalactin (57-kDa major royal jelly proteins) and antiaging[34]. Jellein-I and Jellein-II have broad-spectrum antimicrobial activity, jellein-III is less active, and jellein-IV has no antimicrobial effect (Table 1). Royalisin has antibacterial (gram-positive) activity against *Staphylococcus*, *Streptococcus*, *B. subtilis*, *Micrococcus luteus*, *Sarcina lutea*, *Clostridium*, *Corynebacterium*, *Lactobacillus helveticus*, *Paenibacillus larvae*, and *Leuconostoc*, whereas no inhibition was observed against the gram-negative *E. coli* and *Serratia marcescens*. Antifungal activity against *Botrytis cinerea* was reported for royalisin[35]. In addition, apalbumin 2a (variant of MRJP-2) was found to inhibit the growth of *P. larvae*, *B. subtilis*, and *E. coli* (Table 1).

10-HDA

10-HDA has numerous immunomodulatory activities, such as reduced T cell proliferation, inhibition of interleukin-12 production by spleen dendritic cells, blocking of LPS- and IFN-β-induced NO production in macrophages[36], Th1 stimulation, and Th2 downregulation on human monocyte-derived dendritic cells[37]. 10-HDA was found to protect rats from experimentally induced gastric ulcer[38] and to inhibit LPS-induced NF-κB activation in the murine macrophage cell line RAW264, resulting in an anti-inflammatory effect[16]. 10-HDA has rejuvenated neuron differentiation from rat embryo neural stem cells, probably behaving like the ω-3 docosahexaenoic acid, which is recognized to promote neurogenesis in the central nervous system[39] (Table 1).

Conclusion

AMPs have gained increased attention among researchers, health specialists, and the pharmaceutical industry due to their pharmacological and therapeutic potential. Honey bee AMPs are low molecular weight proteins with a broad range of antimicrobial and immunomodulatory activities against infectious organisms. In addition, honey bee AMPs also exhibit increased efficacy, high specificity, decreased drug interaction, low toxicity, drug delivery shuttles, biological diversity, and direct attacking qualities. Multidrug resistance is multifaceted[40-44], multi-dimensional, and is the second leading cause of death around the world. The urgent need to acquire new antimicrobials has been driving AMPs research. In this regard, AMPs are considered as promising antimicrobial agents for producing a new generation of antimicrobials. Consequently, several pharmaceutical industries are conducting appropriate clinical trials to develop potential therapeutic drugs from AMPs. Over sixty peptide drugs have already been marketed and many novel therapeutic peptides are in preclinical and clinical development. The exploitation of therapeutic AMPs from honey bee sources can also influence novel tactics for the management of human health complications.

Conflict of interest

All authors declare no conflict of interest.

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