The Biotechnological Potential of Bee Venom: Review

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Abstract

In many terrestrial ecosystems, the *Apis mellifera* species played an essential role, being one of the most beneficial insects worldwide. Bee venom (BV) has the role of protecting the bee colony from predators. Among the pharmacological activities of BV are: antibacterial, anti-inflammatory, anticancer, antimutagenic, radioprotective and even antiviral activity. The manifestation of the therapeutic potential is due to the bioactive compounds of BV, the main ones being melittin, phospholipase A_2 and apamin, but hyaluronidase, mast cell degranulation peptide and secapin are also relevant for bioactivity.

The purpose of this paper is to highlight the biotechnological potential, but also the applicability in the medical field as alternative methods to the use of antibiotics.

Keywords: bee venom, biological properties, health applications

1. Introduction

In the bee colony there are many rich reserves of honey, pollen and brood which are targets for a large number of predators. The evolution of insects was due to the use of defense mechanisms against predators. In the bee family, thanks to the high temperature, its constant maintenance and the presence of humidity, the incubation of microorganisms (bacteria, viruses, protozoa and fungi), which most often represent diseases for bees, is facilitated. Due to this consequence, physiological and behavioral adaptations have arisen to counter the increased risks of epidemic diseases [1].

Kuhn-Nentwig (2003) [2] believes that the venom of honey bees (BV) as well as of other hymenoptera is an important source of antimicrobial substances. One method of protection against pathogens is the application of venom to the body surface of bees [3].

BV, also known as apitoxin, is produced in the two abdominal glands (venom gland and dufour gland) of worker bees [4]. The recognition and use of bee venom dates back thousands of years, appearing even in some religious books such as the Bible and the Koran [5]. At the age of 15-20 days, the maximum amount of venom is recorded, approximately 0.30 mg, being influenced by the abundance of food and the breed of bees [6, 7]. As the worker bee ages, the amount of venom decreases. In the case of the queen, the amount of venom is high from the first day to be able to immediately fight with other queens [4]. In contact with air, BV crystallizes and has a white-gray color. Apitoxin has a strong irritating effect, therefore during handling it is necessary to use and respect protective measures [6]. Due to the small size of the bee, extracting a substantial amount of venom is difficult and, to collect it, the bee has to sting, so for this purpose the venom collectors are used [6, 8].

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2. Chemical composition of bee venom

Most insect venoms are composed of peptides, enzymes, proteins and other components. BV has a complex structure comprising peptides, amino acids, proteins, enzymes, biogenic amines, volatile compounds, sugars, phospholipids and pheromones [8]. Structurally, BV is 88% water and 12% other components (Table 1) [9, 10]. The range in which the Ph of bee venom is located is 4.5-5.5 [6].

The results of research undertaken by Bousquet et al. (1979) [11], using the Api-Zym system, identified 55 enzymes present in BV, venom sac, sac-free body extract and commercial whole-body extracts. The components that represent a greater share in the dry weight of BV are melittin 50-60% and PLA2 (phospholipase A_2) 10-12% [8, 10].

Table 1. Processing according to various authors of the major compounds of bee venom

No.	Compounds	Proportion %	Biological action	Authors
			Peptides	
1.	Melittin and isoforms	50-60	Antiviral; Anti-inflammatory; Antifungal; Antibacterial; Anti-atherosclerotic; Pro-apoptotic; Analgesic; Anti-fibrotic; Anti-diabetic; Anti-nociceptive; Antiangiogenic; Wound healing; Haemolysis; Anti-apoptotic; Anti-arthritis; Anti-cancer; Anti-secretory; Anti-arrhythmic	Marques Pereira et al., 2020 [12]; Mohamed et al., 2019 [13]; Yalcin et al., 2009 [14]; Lim et al., 2019 [15]; Jeong et al., 2015 [16]; Kim et al., 2011 [17]; Memariani et al., 2020 [18]; Kong et al., 2016 [19]; Lee et al., 2011 [20]; Choi et al., 2019 [21]; Li et al., 2020 [22]; Khulan et al., 2016 [23]; Hincha et al., 1996 [24]; Shin et al., 2013 [25]; Park et al., 2010 [26]; Sciani et al., 2010 [27]; Tosteson et al., 1987 [28]; Schröder et al., 1971 [29].
2.	Apamin	1-3	Antibacterial; Antifungal; Anti-inflammatory; Anti-atherosclerotic; Anti-cancer; Anti-fibrotic; Neuroprotection	Oršoli´c, 2012 [30]; Kim et al., 2012 [31]; Kim et al., 2017 [32]; Shin et al., 2017 [33]; Lee et al., 2020 [34]; Mohammadi-Rad et al., 2019 [35]
3.	MCD (mast cell-degranulation peptide)	1-3	Anti-allergic; Anti-inflammatory	Buku et al., 2001 [36]; Banks et al., 1990 [37]; Klaudiny, 2007 [38]
4.	Secapin	0.5-2	Antibacterial; Antifungal; Anti-fibrinolytic; Anti-elastolytic	Lee et al., 2016 [39]; Schröder et al., 1971 [29].
5.	Adolapin	0.1-1	Anti-inflammatory; Anti-nociceptive; Antipyretic	Shkenderov et al., 1982 [40]; Wehbe et al., 2019 [41].
			Enzyme	
6.	PLA2 (Phospholipase A_2)	10-12	Antiviral; Inflammatory; Nociceptive; Antibacterial; Neuroprotective; Antigenicity; Allergenicity; Neuronal activation; Nerve regeneration; Anti-cancer; Antiparasitic; Anti-arthritis	Leandro et al., 2015 [42]; Landucci et al., 2000 [43]; Ho et al., 2010 [44]; Dacheux et al., 2019 [45]; Corthésy et al., 2016 [46]; Duchez et al., 2019 [47]; Kim et al., 2019 [48]; Ham et al., 2019 [49]; Shipolini et al., 1974 [50]; Kuchler et al., 1989 [51]
7.	Hyaluronidase	1.5-2	Allergenicity; Spreading factor by hyaluronic acid activation	Markovi´c-Housley et al., 2000 [52]; Csoka et al., 2001 [53]; Gmachl et al., 1993 [54].

The minor compounds of bee venom are dopamine (0.1-1%); acid phosphatase (1%); histamine (0.5-2%); α-glucosidase (0.6%); P, Ca, Mg (3-4%); cardiopep (0.7); minimin (2-3%); pamines (2%); secapin (0.5-2%); phospholipids (1-3%); glucose, fructose (2-4%) and complex ethers (4-8%) [55, 56, 57, 58, 59].

1. Peptides

Melittin (Api m4) has the chemical formula C131H229N39O31 and has a hydrophobic N-

terminus and a hydrophilic C-terminus [8, 60] being a polypeptide containing 26 amino acids. The biological properties of melittin have been studied due to the high degree of participation in the BV composition. Melittin's anti-inflammatory activity is produced by blocking toll-like receptors (TLRs) 2 and 4, cluster of differentiation 14 (CD14) and platelet-derived growth factor receptor beta. Furthermore, melittin has an inhibitory effect on the essential modulator of nuclear factor kappa-B (NF-kB). In the extracellular environment or in the blood vessels, all these pathways end up releasing pro-inflammatory molecules such as inflammatory cytokines, tumor necrosis factors (TNF), nitric oxide (NO) or prostaglandin E2 (PGE2). Inflammatory effects on tissues are produced by these molecules [8, 61].

The ability of melittin to conform pores in biological membranes represents its non-specific cytolytic activity. Anionic lipid membranes attract melittin through its hydrophobic section and positive charge [62]. The combination of a large number of pores can destroy the phospholipid bilayer producing cell lysis [63]. Interaction with cell membranes confers the ability to perform some important biological activities, among them antimicrobial, antifungal, anticancer and hemolytic activity. In the case of fighting infections produced by viruses, melittin has the possibility of being an alternative modality [8].

The results of the research undertaken by Hood et al (2013) [64] revealed that melittin associated with nanoparticles has the ability to inhibit the infectivity of HIV-1 NLHX and HIV-1 NLYU2 viral strains and to deactivate the viral package. Melittin can be a pre-treatment method by stimulating type I interferon (I-IFN) and inhibiting viral replication [65]. Uddin et al. (2016) [65] noted that melittin reduced the amount of virus required to produce cytopathic effect in 50% of inoculated cells.

Melittin interacts with many types of cancer cells, such as inhibiting the growth of ovarian cancer cells [66]. Research by Shin et al. (2013) [67] highlighted anti-angiogenic effects by decreasing the expression of vascular endothelial growth factor (VEGF). Cancer-related mortality is a result of metastases [8].

Apamin is a peptide containing 18 amino acids cross-linked by 2 disulfide bonds, with the molecular formula C83H135N31O26S4 [68]. Blood-brain barrier permeability is a characteristic that gives apamin access to the CNS (central nervous system) [69]. Apamin inhibits cyclooxygenase-2 and decreases the levels of TNF-α, Interleukin-1 (IL-1), IL-6 and NO, conferring apamin its anti-inflammatory property [70]. The results of research undertaken by Kim et al. (2017) [71] showed that apamin is able to suppress Th2-related chemokines and other pro-inflammatory cytokines at the same time as inhibiting the activation of NF-kB (inhibitory effect of nuclear factor kappa-B) and STAT pathways in human keratinocyte cell line.

MCD (mast cell degranulation peptide) or peptide 401 consists of 22 amino acids and has a structure similar to apamin, having 2 bisulfide bridges [72]. MCD is responsible for inflammation, redness and localized pain at the sting site by producing mast cell degranulation and the simultaneous release of histamine [73].

The unique immunological properties of MCD are the release of histamine at concentrations less than 0.1 mg/mL; at higher concentrations it acts as an anti-inflammatory compound [74, 57]. May act as a neurotoxin due to its ability to block Ca2+-activate K+ channels leading to an increase in neuronal excitability [57].

0.5-2% of the dry weight of BV is secapin, which is composed of 25 aa residues and a bisulfide bridge that occurs at the bond between amino acids 9 and 20 [75, 76]. Peptide secapin-1 is similar to serine protease inhibitor that has shown antimicrobial, antifibrinolytic, antifungal and antielastolytic activities [77]. The results of research undertaken by Mourelle et al. (2014) [78] highlighted the hyperalgesic and edematogenic effects of secapin-2.

Adolapin is a basic polypeptide with 103 amino acid residues, representing 0.1-1% of the dry weight of bee venom [75]. Due to inhibition of cyclooxygenase (COX), PLA2 and lipoxygenase, adolapin is anti-inflammatory, anti-nociceptive and antipyretic [79].

Tertiapin is a presynaptic peptide of 21 aa residues with two bisulfide bridges that interacts with rectifier potassium channels expressed in epithelial, cardiac and central nervous system cells by blocking them [80, 81]. In the amino acid sequence, tertiapin has a methionine that triggers chemical changes due to oxidation.

In the composition of bee venom there are other minor constituents such as minimin (2%), procamine A, B (1-2%), pamine and melittin F, a fragment of MLT in which the first seven residues of the N-terminus are missing [82].

2. Enzymes

Phospholipase A2 (Api m1) consists of 134 amino acids, having 5 disulfide bonds, representing 10-12% of the dry weight of BV [83, 81]. Phospholipase A2 (PLA2) in nature has a great diversity, appearing in 16 groups. Bee-derived PLA2 (bPLA2) which is in group III is calcium dependent and has catalytic activity [84]. Research undertaken by Putz et al. (2007) [85] emphasizes membrane-rupturing cytotoxic activity against cancer cells. This cleavage gives bPLA2 antimicrobial activity [86]. The considered major allergen of bee venom is bPLA2 and is proposed as an alternative treatment for neuroinflammatory diseases [87].

Hyaluronidase (Api m2) is composed of 350 amino acids with a disulfide bridge, having the activity of implicitly degrading hyaluronic acid in the extracellular matrix of various tissues, allowing the penetration of venom components into the bloodstream [52]. Because of this ability, hyaluronidase has been given the common name of spreading factor [8]. Hyaluronidase also has other activities related to BV action, cell membrane destruction, pore formation, mast cell degranulation, dilation and increased permeability of blood vessels [9, 81]. In BV, the Api m2 enzyme is the second major allergen, representing 1.5-2% of the dry weight, being able to amplify the action of other toxins in the venom [57].

Acid phosphatase (Api m3) is a potent allergen and a glycoprotein with 4 glycosylation sites [9, 88]. Of the dry weight of BV, acid phosphatase represents 1%, being a stronger stimulant of histamine release from basophils in sensitized humans [57].

Dipeptidyl peptidase IV (DPIV) also called Api m5 represents <1% of the dry weight of bee venom with a molecular weight of 102 kDa [89]. This proteolytic enzyme cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position, which leads to activation of BV components and promotes hypersensitivity reactions [57, 81].

Vitellogenin (Api m12) is a venom allergen, found in greater quantity in the venom of queens, which is involved in several processes such as hormone signaling, feeding behavior, stress resistance, immunity and longevity [81].

3. Biogenic amines

Histamine is the major component of the biogenic amines, representing 0.5-2% of the dry weight of the venom [7]. It causes pain, swelling and itching by promoting capillary leakage [75].

Dopamine and noradrenaline are ionotropic agents and have been shown to be protease inhibitors, playing a role in hemostasis and acting as an anti-inflammatory agent [90].

3. Biological activities

The antimicrobial activity is supported mainly by melittin, which influences biological membranes, but also by PLA 2, which has antimicrobial properties [91]. In the cell membranes of bacteria, damage can be done with vitellogenin [92]. The studies carried out by Memariani et al. (2020) [18] report the antifungal effects of Api m4, and the studies undertaken by Marcos et al. (1995) [93], Wachinger et al. (1992) [94] indicated the antiviral activity of bee venom. Influenza A virus (PR8), herpes simplex virus (HSV), respiratory syncytial virus (RSV) and vesicular stomatitis virus (VSV) reacted to melittin which showed antiviral effects [65].

The antioxidant activity is supported by melittin, apamin and PLA2 by inhibiting the process of lipid peroxidation and increasing the activity of superoxidase dismutase [95]. In mammalian cells via the mechanism of direct cell shielding against oxidative stress, vitellogenin provides cell protection against reactive oxygen species [92]. The results of research undertaken by El-Hanoun et al. (2020) [96] by injecting rabbits with venom twice a week highlighted the antioxidant activity of BV and highlighted the improvement of reproductive performance due to the antioxidant activity of sperm. In the case of rheumatoid arthritis, the research carried out by Kocyigit et al. (2019) [97] highlighted the antioxidant activity of the venom, the results showing that there was no difference between the group treated with low or high doses of BV, the rats increasing their TAS (total plasma antioxidant status) levels and

decreased of TOS (total oxidant status) and OSI (oxidative stress index). The study by Mohamed et al. (2019) [13] in the case of induced gastric ulceration in rats demonstrated the antioxidant activity of BV.

The anti-inflammatory activity is supported by melittin which has been studied against amyotrophic lateral sclerosis, liver inflammation, arthritis, neuroinflammation and acne vulgaris [98]. The most common inflammatory pathology is rheumatoid arthritis, according to studies by Otón et al. (2019) [99], depending on the country, the prevalence is between 0.2 – 0.9 %. The concentration of monosodium urate crystals in the intra-articular area leads to the appearance of gouty arthritis [100].

Neuroprotective activity is supported by PLA2 and apamin, neuroinflammation of chronic activation of microglia and glial cells leads to neurodegenerative disorders, representative diseases being Alzheimer's, Parkinson's and amyotrophic lateral sclerosis [57]. The results of the research undertaken by De Lau et al. (2004) [101] showed that 3 out of 100 people over 65 years of age have Parkinson's disease. bPLA2 has protective effects against inflammatory conditions. Research by Kim et al. (2019) [48] showed that bPLA2 by activating Treg cells reduced inflammation and reduced levels of dopaminergic cell loss, suggesting that PLA2 may be a drug for dopaminergic cell survival.

4. Conclusions

Bee venom is a mixture of substances with biologically active properties, used since ancient times to treat various diseases. Melittin is the most studied and most abundant component. The most allergenic component of BV is considered PLA2 along with histamine. The minor components of the venom still need to be further studied. Most research focuses on immunomodulatory and anti-inflammatory effects. It is important to continue experiments both in vivo and in vitro to see and understand the mechanism of action of bee venom.

References

1.Wilson-Rich, N., Spivak, M., Fefferman, N.H., Starks, P.T., Genetic, individual, and group facilitation of disease resistance in insect societies, Annu. Rev. Entomol., 54, 2009, pp. 405-423, https://doi.org/10.1146/annurev.ento.53.103106.093301

2.Kuhn-Nentwig, L., Peptide antimicrobiene și citolitice ale artropodelor veninoase, CMLS, Celulă. Mol. Life Sci. 60, 2003, 2651–2668, https://doi.org/10.1007/s00018-003-3106-8

3.Baracchi, D., Turillazzi, S., Differences in venom and cuticular peptides in individuals of Apis mellifera (Hymenoptera: Apidae) determined by MALDI-TOF MS, J. Insect Phys., 56, 2010, pp. 366-375, https://doi.org/10.1016/j.jinsphys.2009.11.013

4.Devi, A.B., Sangeeta, Kumar, N.R., Kaur, J., Honey BEE Venom and ITS Composition: Focusing on Different Apis Species -A Review, Journal of Basic and Applied Engineering Research, 2016, 3. 2350-77, https://www.researchgate.net/publication/311615275_Honey_BEE_Venom_and_ITS_Composition_Focusing_on_Different_Apis_Species_-A_Review

5.Wehbe, R., Frangieh, J., Rima, M., El Obeid, D., Sabatier, J. M., & Fajloun, Z., Bee Venom: Overview of Main Compounds and Bioactivities for Therapeutic Interests. Molecules (Basel, Switzerland), 2019, 24(16), 2997, https://doi.org/10.3390/molecules24162997

6.Pătruică S., Biotehnologii în apicultură și sericicultură, Ed. Eurobit, 2021, p.58

7.Moreno, M., Giralt, E., Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: Melittin, Apamin and Mastoparan. Toxins, 2015, 7(4):1126-1150, https://doi.org/10.3390/toxins7041126

8.Carpena, M., Nunez-Estevez, B., Soria-Lopez, A., Simal-Gandara, J., An Updating Review of Its Bioactive Molecules and Its Health Applications, Nutrients, 2020, 2(11):3360, https://doi.org/10.3390%2Fnu12113360

9.Hossen, M.S., Shapla, U.M., Gan, S.H., Khalil, M.I., Impact of Bee Venom Enzymes on Diseases and Immune Responses. Molecules, 2017, 22(1):25, https://doi.org/10.3390/molecules22010025

10.Pucca, M.B., Cerni, F.A., Oliveira, I.S., Jenkins, T.P., Argemí, L., Sørensen, C.V., Laustsen, A.H.,Bee Updated: Current Knowledge on Bee Venom and Bee Envenoming Therapy, Frontiers in Immunology, 2019, 10, https://doi.org/10.3389/fimmu.2019.02090

11.Bousquet, J., Marty, J. P., Clauss, C., Michel, F. B., Enzymes of bee venom, sac and whole body, Annals of allergy, 1979, 43(2), 110–114,https://pubmed.ncbi.nlm.nih.gov/37787/

12.Marques Pereira, A.F., Albano, M., Bérgamo Alves, F.C., Murbach Teles Andrade, B.F., Furlanetto, A., Mores Rall, V.L., Delazari dos Santos, L., de Oliveira Orsi, R., Fernandes, A., Jr., Influence of apitoxin and melittin from Apis mellifera bee on Staphylococcus aureus strains, Microb. Pathog., 2020, 141,104011,https://doi.org/10.1016/j.micpath.2020.104011

13.Mohamed, W.A., Abd-Elhakim, Y.M., Ismail, S.A.A., Involvement of the anti-inflammatory, anti-apoptotic, and anti-secretory activity of bee venom in its therapeutic effects on acetylsalicylic acid-induced gastric ulceration in rats, Toxicology, 2019, 419, 11–23, https://doi.org/10.1016/j.tox.2019.03.003
14.Yalcin, M., Aydin, C., Savci, V., Cardiovascular effect of peripheral injected melittin in normotensive conscious rats: Mediation of the central cholinergic system, Prostaglandins Leukot. Essent. Fat. Acids, 2009, 81, 341–347, https://doi.org/10.1016/j.plefa.2009.10.001
15.Lim, H.N., Baek, S.B., Jung, H.J., Bee venom and its peptide component melittin suppress growth and migration of melanoma cells via inhibition of PI3K/Akt/mTOR and MAPK pathways, Molecules, 2019, 24, 929, https://doi.org/10.3390/molecules24050929
16.Jeong, Y.J., Shin, J.M., Bae, Y.S., Cho, H.J., Park, K.K., Choe, J.Y., Han, S.M., Moon, S.K., Kim, W.J., Choi, Y.H., Kim, C.H., Chang, H.W., Chang, Y.C., Melittin has a chondroprotective effect by inhibiting MMP-1 and MMP-8 expressions via blocking NF- κB and AP-1 signaling pathway in chondrocytes, Int. Immunopharmacol, 2015, 25, 400–405, https://doi.org/10.1016/j.intimp.2015.02.021
17.Kim, S.J., Park, J.H., Kim, K.H., Lee,W.R., Kim, K.S., Park, K.K., Melittin inhibits atherosclerosis in LPS/high-fat treated mice through atheroprotective actions, J. Atheroscler. Thromb, 2011, 18, 1117–1126, https://doi.org/10.5551/jat.8474
18.Memariani, H., Memariani, M., Moravvej, H., Shahidi-Dadras, M., Melittin: A venom-derived peptide with promising anti-viral properties, Eur. J. Clin. Microbiol. Infect. Dis., 2020, 39, 5–17, https://doi.org/10.1007/s10096-019-03674-0
19.Kong, G.M., Tao, W.H., Diao, Y.L., Fang, P.H., Wang, J.J., Bo, P., Qian, F., Melittin induces human gastric cancer cell apoptosis via activation of mitochondrial pathway, World J. Gastroenterol, 2016, 22, 3186–3195, http://dx.doi.org/10.3748/wjg.v22.i11.3186
20.Lee,W.R., Park, J.H., Kim, K.H., Park, Y.Y., Han, S.M., Park, K.K., Protective effects of melittin on transforming growth factor-1 injury to hepatocytes via anti-apoptotic mechanism, Toxicol. Appl. Pharmacol, 2011, 256, 209–215, https://doi.org/10.1016/j.taap.2011.08.012
21.Choi, S., Chae, H.K., Heo, H., Hahm, D.H., Kim, W., Kim, S.K., Analgesic effect of melittin on oxaliplatin-induced peripheral neuropathy in rats, Toxins, 2019, 11, 396, https://doi.org/10.3390/toxins11070396
22.Li, L., Zhang, S., Wei, L., Wang, Z., Ma, W., Liu, F., Shen, Y., Zhang, S., Zhang, X., Hang, Y., Anti-fibrotic effect of melittin on TRIM47 expression in human embryonic lung fibroblast through regulating TRIM47 pathway, Life Sci, 2020, 256, https://doi.org/10.1016/j.lfs.2020.117893
23.Khulan, T.S., Ambaga, M., Effect of Honey bee Venom (Apis mellifera) on Hyperglycemia and Hyperlipidemia in Alloxan Induced Diabetic Rabbits, J. Diabetes Metab., 2016, 6, 3–6, https://doi.org/10.4172/2155-6156.1000507
24.Hincha, D.K., Crowe, J.H., The lytic activity of the bee venom peptide melittin is strongly reduced by the presence of negatively charged phospholipids or chloroplast galactolipids in the membranes of phosphatidylcholine large unilamellar vesicles, Biochim. Biophys. Acta Biomembr, 1996, 1284, 162–170, https://doi.org/10.1016/S0005-2736(96)00122-8
25.Shin, J.M., Jeong, Y.J., Cho, H.J., Park, K.K., Chung, I.K., Lee, I.K., Kwak, J.Y., Chang, H.W., Kim, C.H., Moon, S.K., Melittin Suppresses HIF-1 /VEGF Expression through Inhibition of ERK and mTOR/p70S6K Pathway in Human Cervical Carcinoma Cells, PLoS ONE, 2013, 8, e69380, https://doi.org/10.1371/journal.pone.0069380
26.Park, J.H., Jeong, Y.J., Park, K.K., Cho, H.J., Chung, I.K., Min, K.S., Kim, M., Lee, K.G., Yeo, J.H., Melittin suppresses PMA-induced tumor cell invasion by inhibiting NF- κB and AP-1-dependent MMP-9 expression, Mol. Cells, 2010, 29, 209–215, https://doi.org/10.1007/s10059-010-0028-9
27.Sciani, J.M., Marques-Porto, R., Lourenço Junior, A., Orsi, R. de O., Ferreira Junior, R.S., Barraviera, B., Pimenta, D.C., Identification of a novel melittin isoform from Africanized Apis mellifera venom, Peptides, 2010, 31:1473–9, https://doi.org/10.1016/j.peptides.2010.05.001
28.Tosteson, M.T., Levy, J.J., Caporale, L.H., Rosenblatt, M., Tosteson, D.C., Solidphase synthesis of melittin: purification and functional characterization, Biochemistry, 1987, 26:6627–31, https://doi.org/10.1021/bi00395a010
29.Schröder, E., Lübke, K., Lehmann, M., Beetz, I., Haemolytic activity and action on the surface tension of aqueous solutions of synthetic melittins and their derivatives, Experientia, 1971, 27:764–5, https://doi.org/10.1007/BF02136851
30.Oršoli´c, N., Bee venom in cancer therapy, Cancer Metastasis Rev, 2012, 31, 173–194, https://doi.org/10.1007/s10555-011-9339-3
31.Kim, S.J., Park, J.H., Kim, K.H., Lee, W.R., Pak, S.C., Han, S.M., Park, K.K., The protective effect of apamin on LPS/fat-induced atherosclerotic mice, Evidence-Based Complement. Altern. Med., 2012, 2012, 1–11, https://doi.org/10.1155/2012/305454
32.Kim, J.Y., An, H.J., Kim, W.H., Park, Y.Y., Park, K.D., Park, K.K., Apamin suppresses biliary fibrosis and activation of hepatic stellate cells, Int. J. Mol. Med., 2017, 39, 1188–1194, https://doi.org/10.3892/ijmm.2017.2922

33.Shin, S.H., Ye, M.K., Choi, S.Y., Park, K.K., The effects of melittin and apamin on airborne fungi-induced chemical mediator and extracellular matrix production from nasal polyp fibroblasts. Toxins 2017, 9, 34, https://doi.org/10.3390/toxins9110348
34.Lee, Y.M., Cho, S.N., Son, E., Song, C.H., Kim, D.S., Apamin from bee venom suppresses inflammation in a murine model of gouty arthritis, J. Ethnopharmacol, 2020, 257, 112860, https://doi.org/10.1016/j.jep.2020.112860
35.Mohammadi-Rad, M., Ghasemi, N., Aliomrani, M., Evaluation of apamin effects on myelination process in C57BL/6 mice model of multiple sclerosis, Res. Pharm. Sci., 2019, 14, 424–431, https://doi.org/10.4103/1735-5362.268203
36.Buku, A., Price, J.A., Mendlowitz, M., Masur, S., Mast cell degranulating peptide binds to RBL-2H3 mast cell receptors and inhibits IgE binding, Peptides, 2001, 22, 1993–1998, https://doi.org/10.1016/S0196-9781(01)00542-3
37.Banks, B.E.C., Dempsey, C.E., Vernon, C.A., Warner, J.A., Yamey, J., Anti-inflammatory activity of bee venom peptide 401 (mast cell degranulating peptide) and compound 48/80 results from mast cell degranulation in vivo, Br. J. Pharmacol, 1990, 99, 350–354, https://doi.org/10.1111/j.1476-5381.1990.tb14707.x
38.Klaudiny, J., MRJP9, an ancient protein of the honeybee MRJP family with non-nutritional function, Apicult Res., 2007, 46:99–104, https://doi.org/10.3896/IBRA.1.46.2.06
39.Lee, K.S., Kim, B.Y., Yoon, H.J., Choi, Y.S., Jin, B.R., Secapin, a bee venom peptide, exhibits anti-fibrinolytic, anti-elastolytic, and anti-microbial activities, Dev. Comp. Immunol., 2016, 63, 27–35, https://doi.org/10.1016/j.dci.2016.05.011
40.Shkenderov, S., Koburova, K., Adolapin—A newly isolated analgetic and anti-inflammatory polypeptide from bee venom, Toxicon, 1982, 20, 317–321, https://doi.org/10.1016/0041-0101(82)90234-3
41.Wehbe, R., Frangieh, J., Rima, M., Obeid, D.E., Sabatier, J.M., Fajloun, Z., Bee venom: Overview of main compounds and bioactivities for therapeutic interests, Molecules, 2019, 24, 2997, https://doi.org/10.3390/molecules24162997
42.Leandro, L.F., Mendes, C.A., Casemiro, L.A., Vinholis, A.H.C., Cunha, W.R., De Almeida, R., Martins, C.H.G., Antimicrobial activity of apitoxin, melittin and phospholipase A2 of honey bee (Apis mellifera) venom against oral pathogens., An. Acad. Bras. Cienc., 2015, 87, 147–155, https://doi.org/10.1590/0001-3765201520130511
43.Landucci, E.C.T., Toyama, M., Marangoni, S., Oliveira, B., Cirino, G., Antunes, E., De Nucci, G., Effect of crotapotin and heparin on the rat paw oedema induced by di
erent secretory phospholipases A2, Toxicon, 2000, 38, 199–208, https://doi.org/10.1016/S0041-0101(99)00143-9
44.Ho, J.N., Lee, S.B., Lee, S.S., Yoon, S.H., Kang, G.Y., Hwang, S.G., Um, H.D., Phospholipase A2 activity of peroxiredoxin 6 promotes invasion and metastasis of lung cancer cells, Mol. Cancer Ther., 2010, 9, 825–832, https://doi.org/10.1158/1535-7163.MCT-09-0904
45.Dacheux, M., Sinou, V., Payré, C., Jeammet, L., Parzy, D., Grellier, P., Deregnaucourt, C., Lambeau, G., Antimalarial activity of human group IIA secreted phospholipase A2 in relation to enzymatic hydrolysis of oxidized lipoproteins, Infect. Immun., 2019, 87, 1–18, https://doi.org/10.1128/IAI.00556-19
46.Corthésy, B., Lassus, A., Terrettaz, J., Tranquart, F., Bioley, G., Efficacy of a therapeutic treatment using gas-filled microbubble-associated phospholipase A2 in a mouse model of honeybee venom allergy, Allergy Eur. J. Allergy Clin. Immunol., 2016, 71, 957–966, https://doi.org/10.1111/all.12859
47.Duchez, A.C., Boudreau, L.H., Naika, G.S., Rousseau, M., Cloutier, N., Levesque, T., Gelb, M.H., Boilard, E., Respective contribution of cytosolic phospholipase A2
and secreted phospholipase A2 IIA to inflammation and eicosanoid production in arthritis, Prostaglandins Other Lipid Mediat., 2019, 143, 106340, https://doi.org/10.1016/j.prostaglandins.2019.106340
48.Kim, K.H., Lee, S.Y., Shin, J., Hwang, J.T., Jeon, H.N., Bae, H., Dose-dependent neuroprotective effect of standardized bee venom phospholipase A2 against MPTP-induced Parkinson's disease in mice, Front. Aging Neurosci., 2019, 11, 1–9, https://doi.org/10.3389/fnagi.2019.00080
49.Ham, H.J., Han, J.H., Lee, Y.S., Kim, K.C., Yun, J., Kang, S.K., Park, Y.S., Kim, S.H., Hong, J.T., Bee Venom Soluble Phospholipase A2 Exerts Neuroprotective Effects in a Lipopolysaccharide-Induced Mouse Model of Alzheimer's Disease via Inhibition of Nuclear Factor-Kappa B, Front. Aging Neurosci., 2019, 11, 1–14, https://doi.org/10.3389/fnagi.2019.00287
50.Shipolini, R.A., Callewaert, G.L., Cottrell, R.C., Vernon, C.A., The amino-acid sequence and carbohydrate content of phospholipase A2 from bee venom, Eur J Biochem, 1974, 48:465–76, https://doi.org/10.1111/j.1432-1033.1974.tb03787.x
51.Kuchler, K., Gmachl, M., Sippl, M.J., Kreil, G., Analysis of the cDNA for phospholipase A2 from honeybee venom glands. The deduced amino acid sequence reveals homology to the corresponding vertebrate enzymes, Eur J Biochem., 1989, 184:249–54, https://doi.org/10.1111/j.1432-1033.1989.tb15014.x
52.Markovi´c-Housley, Z., Miglierini, G., Soldatova, L., Rizkallah, P.J., Müller, U., Schirmer, T., Crystal

structure of hyaluronidase, a major allergen of bee venom, Structure, 2000, 8, 1025–1035, https://doi.org/10.1016/S0969-2126(00)00511-6
53.Csoka, A.B., Frost, G.I., Stern, R., The six hyaluronidase-like genes in the human and mouse genomes, Matrix Biol., 2001, 20, 499–508, https://doi.org/10.1016/S0945-053X(01)00172-X
54.Gmachl M., Kreil G., Bee venomhyaluronidase is homologous to a membrane protein of mammalian sperm, Proc Natl Acad Sci USA, 1993, 90:3569, https://doi.org/10.1073/pnas.90.8.3569
55.Van Vaerenbergh, M., Debyser, G., Devreese, B., de Graaf, D.C., Exploring the hidden honeybee (Apis mellifera) venom proteome by integrating a combinatorial peptide ligand library approach with FTMS, J. Proteom, 2014, 99, 169–178, https://doi.org/10.1016/j.jprot.2013.04.039
56.Abd El-Wahed, A.A., Khalifa, S.A.M., Sheikh, B.Y., Farag, M.A., Saeed, A., Larik, F.A., Koca-Caliskan, U., AlAjmi, M.F., Hassan, M., Wahabi, H.A., et al., Bee Venom Composition: From Chemistry to Biological Activity, Stud. Nat. Prod. Chem., 2018, 60, 459–484, https://doi.org/10.1016/B978-0-444-64181-6.00013-9
57.Pascoal, A., Estevinho, M.M., Choupina, A.B., Sousa-Pimenta, M., Estevinho, L.M., An overview of the bioactive compounds, therapeutic properties and toxic effects of apitoxin, Food Chem. Toxicol, 2019, 134,110864, https://doi.org/10.1016/j.fct.2019.110864
58.Chen, J., Lariviere, W.R., The nociceptive and anti-nociceptive effects of bee venom injection and therapy: A double-edged sword, Prog. Neurobiol., 2010, 92, 151–183, https://doi.org/10.1016/j.pneurobio.2010.06.006
59.Son, D.J., Lee, J.W., Lee, Y.H., Song, H.S., Lee, C.K., Hong, J.T., Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds, Pharmacol. Ther., 2007, 115, 246–270, https://doi.org/10.1016/j.pharmthera.2007.04.004
60.National Biotechnology Information Center, PubChem Compound Summary for CID 16133648, Melittin, 2022, Retrieved 25, November 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/Melittin
61.Sun, X., Chen, S., Li, S., Yan, H., Fan, Y., Mi, H., Deletion of two C-terminal Gln residues of 12–26-residue fragment of melittin improves its antimicrobial activity, Peptides, 2005, 26, 369–375, https://doi.org/10.1016/j.peptides.2004.10.004
62.Klocek, G., Schulthess, T., Shai, Y., Seelig, J., Thermodynamics of Melittin Binding to Lipid Bilayers, Aggregation and Pore Formation. Biochemistry, 2009, 48, 2586-2596, https://doi.org/10.1021/bi802127h
63.Lee, M.T., Sun, T.L., Hung,W.C., Huang, H.W., Process of inducing pores in membranes by melittin, Proc, Natl. Acad. Sci, 2013, 110, 14243–14248, https://doi.org/10.1073/pnas.1307010110
64.Hood, J.L., Jallouk, A.P., Campbell, N., Ratner, L., Wickline, S.A., Cytolytic nanoparticles attenuate HIV-1 infectivity, Antiviral therapy, 2013, 18(1):95-103, https://doi.org/10.3851/IMP2346
65.Uddin, M.B., Lee, B.H., Nikapitiya, C., Kim, J.H., Kim, T.H., Lee, H.C., Kim, C.G., Lee, J.S., Kim, C.J., Inhibitory effects of bee venom and its components against viruses in vitro and in vivo, J. Microbiol., 2016, 54, 853–866, https://doi.org/10.1007/s12275-016-6376-1
66.Jo, M., Park, M.H., Kollipara, P.S., An, B.J., Song, H.S., Han, S.B., Kim, J.H., Song, M.J., Hong, J.T., Anti-cancer efect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway, Toxicol, Appl. Pharmacol, 2012, 258, 72–81, https://doi.org/10.1016/j.taap.2011.10.009
67.Shin, J.-M., Jeong, Y.-J., Cho, H.-J., Park, K.-K., Chung, I.-K., Lee, I.-K., Chang, Y.-C., Melittin Suppresses HIF-1α/VEGF Expression through Inhibition of ERK and mTOR/p70S6K Pathway in Human Cervical Carcinoma Cells, PLOS ONE, 2013, 8(7), https://doi.org/10.1371/journal.pone.0069380
68.National Biotechnology Information Center, PubChem Compound Summary for CID 91808948, Apamin, N-acetyl-4-(N6-acetyl-L-lysine)-, 2022, Retrieved November 29, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/Apamin_-N-acetyl-4-_N6-acetyl-L-lysine
69.Palma, M. S., Chapter 58 - Hymenoptera Insect Peptides, In A. J. Kastin (Ed.), Handbook of Biologically Active Peptides (Second Edition), 2013, pp. 416-422, Boston: Academic Press, https://doi.org/10.1016/B978-0-12-385095-9.00058-0
70.Shin, S. H., Ye, M. K., Choi, S. Y., & Park, K. K., Anti-inflammatory effect of bee venom in an allergic chronic rhinosinusitis mouse model, Molecular medicine reports, 2018, 17(5), 6632–6638, https://doi.org/10.3892/mmr.2018.8720
71.Kim, W. H., An, H. J., Kim, J. Y., Gwon, M. G., Gu, H., Lee, S. J., Park, J. Y., Park, K. D., Han, S. M., Kim, M. K., Park, K. K., Apamin inhibits TNF-α- and IFN-γ-induced inflammatory cytokines and chemokines via suppressions of NF-κB signaling pathway and STAT in human keratinocytes, Pharmacological reports : PR, 2017, 69(5), 1030–1035. https://doi.org/10.1016/j.pharep.2017.04.006
72.Ziai, M. R., Russek, S., Wang, H.-C., Beer, B., Blume, A. J., Mast Cell Degranulating Peptide: A Multi-functional Neurotoxin, Journal of Pharmacy and Pharmacology, 2011, 42(7), 457-461, https://doi.org/10.1111/j.2042-7158.1990.tb06595.x
73.Buku, A., Price, J.A., Further studies on the structural requirements for mast cell degranulating (MCD) peptide-mediated histamine release, Peptides,

2001, 22, 1987–1991, https://doi.org/10.1016/S0196-9781(01)00538-1
74.Moga, M.A., Dimienescu, O.G., Arvătescu, C.A., Ifteni, P., Pleș, L., Anticancer activity of bee and snake venom toxins, An overview of ovarian cancer, Molecules, 2018, 23, 692, https://doi.org/10.3390/molecules23030692
75.Rady, I., Siddiqui, I. A., Rady, M., & Mukhtar, H., Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy, Cancer Letters, 2017, 402, 16-31, https://doi.org/10.1016/j.canlet.2017.05.010
76.Meng, Y., Yang, X.X., Sheng, Y.X., Zhang, J.L., Yu, D.Q., A novel peptide from Apis mellifera and solid-phase synthesis of its analogue. Chin. Chem. Lett., 2012, 23, 1161–1164, https://doi.org/10.1016/j.cclet.2012.09.003
77.Lee, G., Bae, H., Anti-inflammatory applications of melittin, a major component of bee venom: Detailed mechanism of action and adverse effects, Molecules, 2016, 21, 616, https://doi.org/10.3390/molecules21050616
78.Mourelle, D., Brigatte, P., Bringanti, L.D.B., De Souza, B.M., Arcuri, H.A., Gomes, P.C., Baptista-Saidemberg, N.B., Ruggiero Neto, J., Palma, M.S., Hyperalgesic and edematogenic effects of Secapin-2, a peptide isolated from Africanized honeybee (Apis mellifera) venom, Peptides, 2014, 59, 42–52, https://doi.org/10.1016/j.peptides.2014.07.004
79.Elieh Ali Komi D., Shafaghat F., Zwiener R.D., Immunology of bee venom, Clinical Reviews in Allergy and Immunology, 2018, 54(3):386-396, https://doi.org/10.1007/s12016-017-8597-4
80.Kitamura, H., Yokoyama, M., Akita, H., Matsushita, K., Kurachi, Y., Yamada, M., Tertiapin potently and selectively blocks muscarinic K(+) channels in rabbit cardiac myocytes, The Journal of pharmacology and experimental therapeutics, 2000, 293(1), 196–205, https://pubmed.ncbi.nlm.nih.gov/10734170/
81.Dos Santos-Pinto, J.R.A., Perez-Riverol, A., Lasa, A. M., Palma, M. S., Diversity of peptidic and proteinaceous toxins from social Hymenoptera venoms, Toxicon, 2018, 148, 172-196, https://doi.org/10.1016/j.toxicon.2018.04.029
82.Chen, J., Guan, S.-M., Sun, W., Fu, H., Melittin, the Major Pain-Producing Substance of Bee Venom, Neuroscience Bulletin, 2016, 32(3), 265-272, https://doi.org/10.1007/s12264-016-0024-y
83.Welker, S., Markert, Y., Köditz, J., Mansfeld, J., Ulbrich-Hofmann, R., Disulfide bonds of phospholipase A2 from bee venom yield discrete contributions to its conformational stability, Biochimie, 2011, 93(2), 195-201, https://doi.org/10.1016/j.biochi.2010.09.012
84.Dennis, E.A., Cao, J., Hsu, Y.H., Magrioti, V., Kokotos, G., Phospholipase A2 enzymes: Physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention, Chem. Rev., 2011, 111, 6130–6185, https://doi.org/10.1021/cr200085w
85.Putz, T., Ramoner, R., Gander, H., Rahm, A., Bartsch, G., Bernardo, K., Ramsay, S., Thurnher, M., Bee venom secretory phospholipase A2 and phosphatidylinositol-homologues cooperatively disrupt membrane integrity, abrogate signal transduction and inhibit proliferation of renal cancer cells. Cancer Immunol, Immunother. 2007, 56, 627–640, https://doi.org/10.1007/s00262-006-0220-0
86.Samy, R.P., Gopalakrishnakone, P., Thwin, M.M., Chow, T.K.V., Bow, H., Yap, E.H., Thong, T.W.J., Antibacterial activity of snake, scorpion and bee venoms: A comparison with purified venom phospholipase A2 enzymes, J. Appl. Microbiol., 2007, 102, 650–659, https://doi.org/10.1111/j.1365-2672.2006.03161.x
87.Chung, E.S., Lee, G., Lee, C., Ye, M., Chung, H., Kim, H., Bae, S.S., Hwang, D.-S., Bae, H., Bee Venom Phospholipase A 2, a Novel Foxp3 + Regulatory T Cell Inducer, Protects Dopaminergic Neurons by Modulating Neuroinflammatory Responses in a Mouse Model of Parkinson's Disease, J. Immunol., 2015, 195, 4853–4860, https://doi.org/10.4049/jimmunol.1500386
88.Grunwald, T., Bockisch, B., Spillner, E., Ring, J., Bredehorst, R., Ollert, M.W., Molecular cloning and expression in insect cells of honeybee venom allergen acid phosphatase (Api m 3), J. Allergy Clin. Immunol., 2006, 117, 848–854, https://doi.org/10.1016/j.jaci.2005.12.1331
89.Blank, S., Seismann, H., Bockisch, B., Braren, I., Cifuentes, L., McIntyre, M., Rühl, D., Ring, J., Bredehorst, R., Ollert, M.W., Grunwald, T., Spillner, E., Identification, Recombinant Expression, and Characterization of the 100 kDa High MolecularWeight Hymenoptera Venom Allergens Api m 5 and Ves v 3. J, Immunol., 2010, 184, 5403–5413, https://doi.org/10.4049/jimmunol.0803709
90.Nitecka-Buchta, A., Buchta, P., Tabeńska-Bosakowska, E., Walczyńska-Dragoń, K., Baron, S., Myorelaxant Effect of Bee Venom Topical Skin Application in Patients with RDC/TMD Ia and RDC/TMD Ib: A Randomized, Double Blinded Study, BioMed Research International, 2014, 296053, https://doi.org/10.1155/2014/296053
91. Al-Ani, I., Zimmermann, S., Reichling, J., Wink, M., Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens, Phytomedicine, 2015, 22, 245–255, https://doi.org/10.1016/j.phymed.2014.11.019
92.Park, H.G., Lee, K.S., Kim, B.Y., Yoon, H.J., Choi, Y.S., Lee, K.Y., Wan, H., Li, J., Jin, B.R., Honeybee (Apis cerana) vitellogenin acts as an antimicrobial and

antioxidant agent in the body and venom, Dev. Comp. Immunol., 2018, 85, 51–60, https://doi.org/10.1016/j.dci.2018.04.001
93.Marcos, J.F., Beachy, R.N., Houghten, R.A., Blondelle, S.E., Pérez-Payá, E., Inhibition of a plant virus infection by analogs of melittin, Proc. Natl. Acad. Sci. USA, 1995, 92, 12466–12469, https://doi.org/10.1073/pnas.92.26.12466
94.Wachinger, M.; Saermark, T.; Erfle, V. Influence of amphipathic peptides on the HIV-1 production in persistently infected T lymphoma cells, FEBS Lett, 1992, 309, 235–241, https://doi.org/10.1016/0014-5793(92)80780-K
95.Sobral, F., Sampaio, A., Falcão, S., Queiroz, M.J.R.P., Calhelha, R.C., Vilas-Boas, M., Ferreira, I.C.F.R., Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal, Food Chem., Toxicol., 2016, 94, 172–177, https://doi.org/10.1016/j.fct.2016.06.008
96.El-Hanoun, A., El-Komy, A., El-Sabrout, K., Abdella, M., Effect of bee venom on reproductive performance and immune response of male rabbits, Physiol. Behav., 2020, 223, 112987, https://doi.org/10.1016/j.physbeh.2020.112987
97.Kocyigit, A., Guler, E.M., Kaleli, S., Anti-inflammatory and antioxidative properties of honey bee venom on Freund's Complete Adjuvant-induced arthritis model in rats, Toxicon, 2019, 161, 4–11, https://doi.org/10.1016/j.toxicon.2019.02.016
98. .Lee, G., Bae, H., Anti-inflammatory applications of melittin, a major component of bee venom: Detailed mechanism of action and adverse effects, Molecules, 2016, 21, 616, https://doi.org/10.3390/molecules21050616
99.Otón, T., Carmona, L., The epidemiology of established rheumatoid arthritis, Best Pr. Res. Clin. Rheumatol, 2019, 33, 3–12, https://doi.org/10.1016/j.berh.2019.101477c
100.Davies, J., Riede, P., van Langevelde, K., Teh, J., Recent developments in advanced imaging in gout, Ther. Adv. Musculoskelet. Dis., 2019, 11, 1759720X1984442, https://doi.org/10.1177/1759720X19844429
101.De Lau, L.M.L., Giesbergen, P.C.L.M., De Rijk, M.C., Hofman, A., Koudstaal, P.J., Breteler, M.M.B., Incidence of parkinsonism and Parkinson disease in a general population: The Rotterdam Study. Neurology, 2004, 63, 1240–1244, https://doi.org/10.1212/01.WNL.0000140706.52798.BE