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REVIEW ARTICLE

BEE VENOM – FRIEND OR ENEMY

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Summary

Increasing resistance to antibiotics, adverse effects of standard anti-cancer or anti-inflammatory treatments, or tumour types resistant to these treatments are leading to a search for alternatives. One of these is the use of natural products, such as bee venom, which have the same or better effect than these standard products. Bee venom has been used to treat a number of diseases for thousands of years. However, a significant obstacle remains the risk of severe allergic reactions, which can be caused by some of the more than 100 substances contained in the venom. Therefore, intensive research is currently underway to investigate not only the actual use of bee venom or its components in the above areas, but also ways to prevent these adverse effects.

Key words: bee; venom; apitherapy; allergic reaction; api m; envenoming

Introduction

Natural substances have been used in medicine for thousands of years. This is also the case with products created by bees. According to various sources, the roots of apitherapy date back to 3000 to 6000 BC (1,2). The first surviving references to the use of bee venom as a medicine in Europe are attributed to Hippocrates (460-370 BC), who used bee venom (BV) to treat baldness. In the 15th century at the court of Ivan the Terrible, bee venom was used to treat other ailments such as gout. Dr. Filip Terč, a physician with Czech roots, is considered to be the founder of modern apitherapy and used bee venom to treat rheumatism (3,4). The discovery of antibiotics in the 20th century reduced the interest in natural substances, but their overuse and the associated increase in resistance to them led scientists back to the search for substances that could be used not only in the treatment of infectious diseases. One of these substances is bee venom. The aim of the authors is to summarise the current knowledge of bee venom, present its composition, effects and risks associated with its potential use, including ways to reduce these risks and areas where the effects of bee venom are being intensively tested (see Figure 1,2,3), both *in vitro* and *in vivo* and in potential clinical trials.

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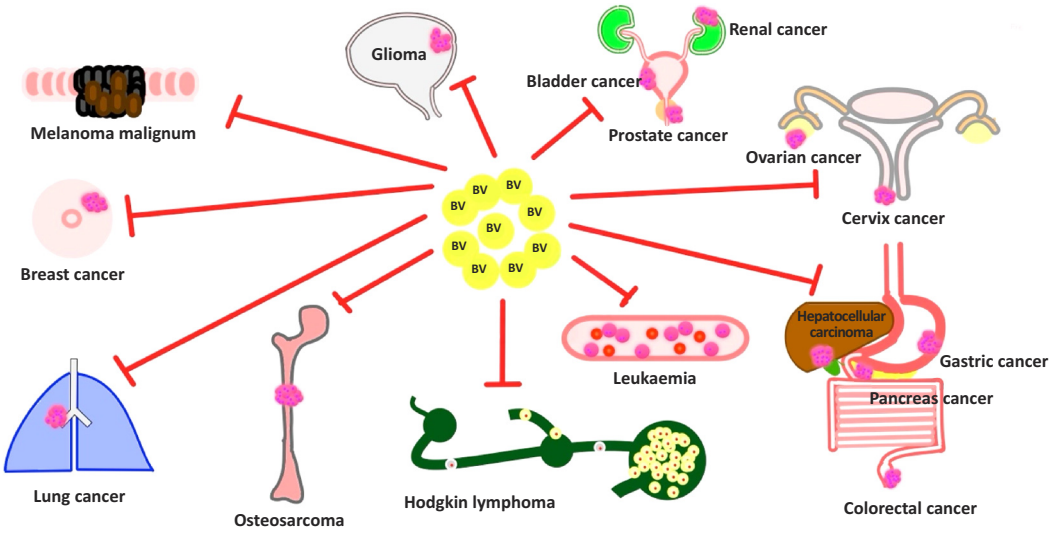

Figure 1. Schematic overview of the anticancer activity of bee venom as published in the scientific literature.

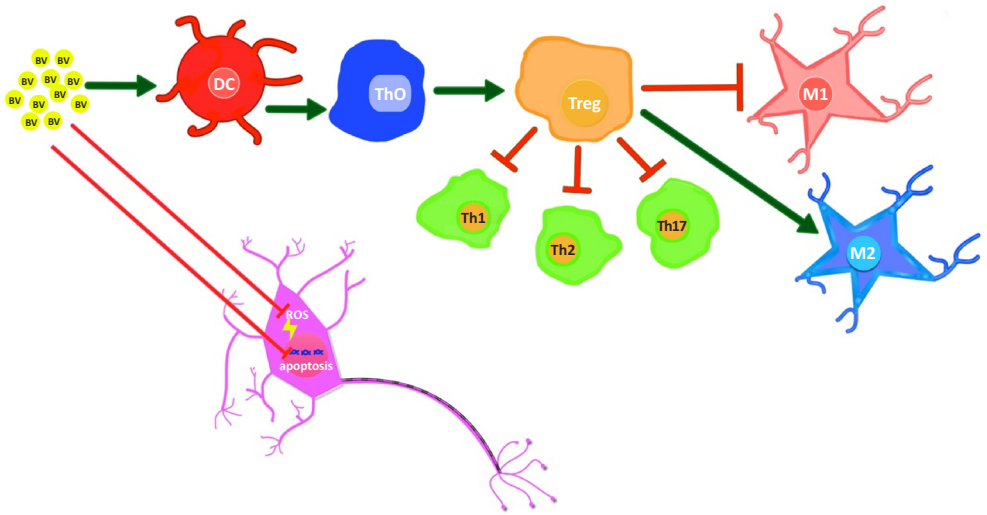

Figure 2. A simplified representation of the neuroprotective effect of honey bee venom in neurodegenerative diseases.

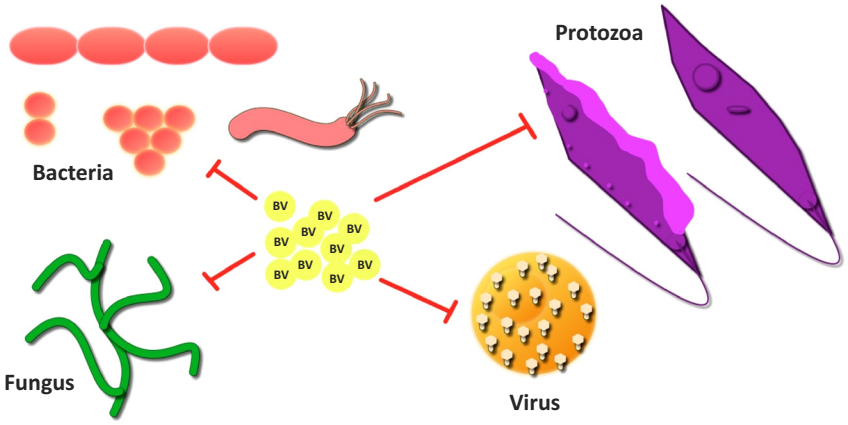

Figure 3. The antimicrobial effect of bee venom on the most common pathogens.

General properties of bee venom

BV is a clear, odorless, acidic liquid (pH 4,5-5,5) with a bitter taste and sometimes a natural pungent odor that forms in the venom gland that only queens and workers have. This fluid is a mixture of 113 different substances (5–7), including enzymes, peptides, biogenic amines, amino acids, sugars, pheromones, and minerals where water makes up 80-88 % of the volume of bee venom, according to various works (8). Bee venom is soluble in water, but not in alcohol or ammonium sulfate. It easily dries up at room temperature during which forms grey-white crystals. Dried BV has pale yellow color, brown in the case of the commercially advanced form, probably due to oxidation of some of its proteins (5,8,9). The excess production of venom gland is stored in the venom sac, from where it is then injected by the sting apparatus into the sting site. When stung, the bee may not empty the entire venom sac (0,15-0,30 mg). This usually occurs when the entire sting apparatus (venom sac, muscle, nerve ganglion) is lost, which continues to pump venom into the wound for some time after the sting (9). The composition and amount of venom is the same across representatives of the Apis species. However, they differ slightly in production and toxicity due to their physiological differences (10).

The activity of the venom gland is influenced by a number of internal and external factors.

Internal factors represent: 1) Strain - the Eastern honey bee (Apis cerana), which is found mainly in Asia (India, China, Korea, Indonesia) (11), produces about twice as much venom as the European honey bee (Apis mellifera) (12). The amount of mellittin and hyaluronidase in the African honey bee venom is less than in the European strains, on the other hand contains more phospholipase A2 (PLA2). European honey bee also releases about five times more venom than the African honey bee. 2) Caste – the venom produced by the workers contains more mellitin and apamin than the queen venom while the opposite is true for the histamin. 3) Age – the production of venom increases during the first two weeks of the bee's life and reaches a maximum when the bee is engaged in hive defence and foraging. Age affects not only the amount of venom but also its composition. While the melittin content increases continuously until the 4th week of the bee's life and then gradually decreases, the PLA2 content reaches its maximum level between 7-10 days after enclosion and remains constant (7).

External factors include the period (melittin and PLA2 reach a maximum in March and May and a minimum in January (7) and methods of venom acquisition. According to the work of Hsiang and Elliott (1975), protein content of venom obtained by surgical removal of venom sac was different from that obtained by electrical milking. Ferreira Junior (2009) confirmed the same a few decades later. Pence (1981), based on his studies, found that the most potent venom seems to come from poisons collected underwater to prevent evaporation of certain volatiles. And Kumar and Devi (2014) found differences in the composition of venom gland secretions and venom sacs that were surgically removed in workers of different species of Apis spieces (7,10,13–15).

Classification, properties of bee venom allergens

As mentioned above, bee venom is a mixture of at least 113 substances identified so far, 12 of which are registered in the WHO/IUIS database as Api m 1 - Api m 12 allergens. These allergens have different biological functions, which are listed in the Table 1.

Table 1.

Alergen	Mechanism of Action
Api m 1 (Phospholipase A2)	mediates the disruption of cellular membranes, pore formation, necrosis, realising lysophospholipids and fatty acids (16–18) causes hemolysis, platelet aggregation, local edema formation, cell necrosis, massive liberation of pro-inflamatory mediators (19–24)
Api m 2 (Hyaluronidase)	hydrolyses hyalluronic acid (HA), these particles have pro-inflamatory, immunostimulating, pro-angiogenic capabilities (25) breaks down glycolide linkages, decreases viscosity of the tissue, allows penetration of the venom into the tissues, stimulates blood vesel dilatation and increases permeability (26,27)

Alergen	Mechanism of Action
Api m 3 (Venom acid phosphatase)	capable of release histamine, trigger wheal-and-flare reaction (swelling/redness) (28–30)
Api m 4 (Melittin)	non-selective peptide that causes disruption of all prokaryotic and eukaryotic cell membranes, where binding to their negatively charged surface results in the formation of pores in the phospholipid bilayer, leading to leakage of intracellular atomic ions and molecules, increasing their permeability and ultimately lysis. (31–36)
Api m 5 (Dipeptidylpeptidase IV)	stepwise split promelittin into melittin (37,38) modulates the chemotactic activity of immune cells after the insect sting (39)
Api m 6 (Serine protease inhibitor)	recombinant induces damage to the bacterial and fungal cell walls and has inhibitory effects on trypsin, plasmin and microbial serine protease (40)
Api m 7 (Serine proteinase)	serine protease kills target insects via a melanization responce, in mammals activates prothrombin (41)
Api m 8 (Carboxylesterase)	Bumblebee venom carboxylesterase degrades triglycerides (42) key enzymes of pesticide detoxification in insects (43)
Api m 9 (Serine carboxypeptidase)	not found
Api m 10 (Icarapin)	not found
Api m 11 (Major royal jelly protein 8,9)	inhibits microbial serine protease (44)
Api m 12 (Vitellogenin)	binds to microbial surfaces and causes structural damage to microbial cell walls (45)

Classification of allergic reactions and their severity

The venom of Hymenoptera (bees, wasps) is one of the three most common causes of anaphylactic reactions worldwide, along with drugs and food (46). The prevalence in the adult population is 7,5 % in Europe and 3,3 % in the USA (47,48). Approximately 15,5 – 42,0 % of these venom-induced reactions are rated as severe (49–56). This variance is probably due to the lack of a universally used system for assessing the severity of acute allergic reactions (Table 2). If we assume a 3 % prevalence of individuals in the population with known allergy to bee venom, then the recently estimated mortality rate is less than 1/100,000 per year. In reality, it will be higher (57). Thus, the goal is to identify the maximum number of patients allergic to Hymenoptera venom. Unfortunately, up to 60 % of fatal reactions occur in people who previously did not know they were allergic to insect venom (58).

There are a number of factors that can aggravate the course of an allergic reaction. These factors may reduce the dose of allergen required to induce an anaphylactic reaction, either by lowering the threshold for mast cell activation or by increasing the availability of the allergen. These factors can be divided into 2 groups. The first group consists of external factors. These include, for example, delayed adrenaline administration, upright posture, physical activity during or just before the anaphylactic reaction. And intrinsic factors including mainly age (according to a European multicentre study, there is a linear relationship between increasing age and the severity of allergic reaction to stings, whereas anaphylactic reaction caused by stings is not common in children), mastocytosis (especially indolent systemic mastocytosis), male gender (probably as a consequence of more frequent exposure to stings), elevated basal serum tryptase, absence of skin symptomes during anaphylaxis, short time interval between sting and onset of symptoms (49,51,58,62–64).

Diagnostic possibilities of hypersensitivity to bee venom

Although history experience and a lot of current *in vitro* and *in vivo* studies prove the usefulness of many BV compounds, the main obstacles prevent wide use are safety and adverse effects of some of these components. To manage maximum safety during application of BV products is necessary to reduce the possibility of adverse effects, mainly the most serious.

Table 2.

Clasification of systemic reaction according to:			
	MÜLLER (59)	**RING AND MESSMER** (60)	**BRITISH SOCIETY FOR ALLERGY AND CLINICAL IMMUNOLOGY** (61)
I/mild	Anxiety, itching, urticaria, malaise	Generalized skin symptoms (flush, generalized urticaria, angioedema)	Pruritus, urticaria, erythema, mild angioedema, rhinitis, conjuctivitis
II/moderate	Any of the above plus two or more of the following: angioedema (grade II, also if alone), constriction in chest, nauzea, vomiting, diarrhoea, abdominal pain, dizziness	Mild to moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms	Mild asthma, moderate angioedema, abdominal pain, vomiting, diarrhoea and minor or transient hypotensive symptoms such as lightheadedness and dizzines
III/severe	Any of the above plus two or more of the following: dyspnoea, wheezing, stridor (any of these alone are grade III), dysphagia, dysarthria, hoarseness, weakness, confusion, feeling of impending disaster	Anaphylactic shock, loss of consciousness	Respiratory difficulty such as asthma or laryngeal oedema, hypotension, collapse or loss of consciousness, as well as double incontinence, seizures, or loss of colour vision
IV	Any of the above plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence (urine, stool), cyanosis	Cardiac arrest, apnoea	Anaphylaxis – severe, life-threatening, generalised or systemic hypersensitivity reaction

This can be achieved in several ways:

1) By selecting the patient based on a thorough history, focusing on the severity of previous insect bites and the causative agent of the reaction. This should be a focal point given that current recommendations state that a diagnosis of allergy to whitefly venom is only indicated in persons who have a history of a previous systemic reaction following an insect bite (63,65,66). The reason for this is the high prevalence of sensitization to whitefly venom, which is not clinically significant in a large proportion of the general population and is approximately 27-40 %; in children, it is as high as 50 %. The risk of a systemic reaction in an asymptomatic sensitized individual is very low at approximately 5,3 % (67–69).

The risk of a systemic reaction in the general population in individuals who have a history of a large local reaction to a sting (swelling greater than 10 cm in diameter, lasting more than 24 hours, prevalence in the general population is 26 %) is also low, reaching 10 %. In these individuals, sensitization to venom is as high as 80 %. Similarly, the development of a systemic reaction is not very common in patients who have experienced serum sickness-like reactions or toxin reactions due to a large number of stings (66,70).

In addition to the origin of the venom, up to 50 % of patients allergic to the venom of whiteflies are simultaneously sensitized to both Honey Bee Venom (HBV) and Yellow Jacket Venom (YJV), but usually only one of them is clinically significant (71).

2) Skin tests that are quick, simple and inexpensive. Tests are performed with gradually increasing concentrations of venom until either a positive reaction or the recommended maximum test concentration is achieved. Commercially available standardized venom extracts are used for the tests. In the event of a negative Prick test result (maximum dose is 300 μg/ml), intradermal tests are added, again performed with gradually increasing doses of venom (maximum dose is 1 μg/ml). Intradermal tests increase the sensitivity of skin tests to insect venom from 67-74 % to 95-98 % (66,71-73).

Because of the possibility of false-negative results due to tachyphylaxis, it is advisable to avoid testing patients shortly after an insect bite. On the contrary, the period 1-6 weeks after the sting appears to be the most optimal period, probably due to the high number of venom-specific IgE antibodies. On the other hand, there is a 12 %

reduction in skin test sensitivity one year after the bite and 33 % are negative at two and a half years (74-76). It should be noted that there are a number of substances that can affect skin test results, and these substances should be discontinued prior to testing. Examples include first and second-generation antihistamines, glucocorticosteroids, benzodiazepines, omalizumab, tricyclic antidepressants, and some neuroleptics (76).

3) Determination of specific IgE antibodies to whole venom preparations. In most cases, specific IgE antibodies to both bee and wasp venom are tested simultaneously. Due to the high similarity of wasp and hornet venom (approximately 95 %), this test is sufficient even in patients who have had a hornet sting in the past (72-73). In patients allergic to bee venom, the sensitivity of sIgE for HBV is 98-100 %, whereas for wasp venom, the sensitivity of sIgE for YJV is 83-93 % (75,77–78). Similar to skin testing, the most appropriate time for IgE antibody collection is 1-6 weeks after the sting (65,79-81), as the concentration of these antibodies also declines, especially in the 1-4 years following an anaphylactic reaction to Hymenoptera venom. It is important to add that antibody levels may fall below detectable levels in the long term (76). Currently 12 bee allergens (Apis mellifera, cerana, dorsata), 6 Yellow Jacket allergens (Vespula vulgaris, flavopilosa, germanica, maculifrons, pensylvanica, squamosa, vidua), 2 Bumblebee allergens (Bombus pensylvanicus, terrestris), 3 White-faced hornet, Yellow hornet allergens (Dolichovespula maculate, arenaria), 2 Hornet allergens (Vespa crabro, magnifica, mandarinia), 3 European paper wasp allergens (Polistes dominula, gallicus) and 4 American paper wasps allergens (Polistes annularis, exclamans, fuscatus, metricus) have been described (76-77,81). Higher levels of total IgE (>250 kU/l) may be associated with a milder course of allergic reaction after a sting in patients with insect venom allergy (55).

Skin tests, together with the determination of specific antibodies to Hymenoptera venom, are standard, long-used methods for the detection of insect venom allergy that have good sensitivity but are burdened by a high percentage of double positivity. This means that 30-60 % of patients tested in this way have simultaneous allergy to bee and wasp venom (75). In some patients, the results actually reflect the reality of double sensitization after wasp and bee stings, but in most they do not. The cause of this false double positivity is cross-reactivity between insect venoms. There are several reasons for this – protein-specific cross-reactivity due to sensitization of the patient to highly homologous allergic components contained in both wasp and bee venom (hyaluronidase, dipeptidyl peptidase IV, vitellogenins). The second and more common reason is IgE-mediated sensitization to CCD (cross-reactive carbohydrate determinant), which accounts for up to 50 % of dual sensitization to bee and wasp venom (76,82).

4) Molecular diagnostics and its methods have made it possible to create recombinant forms of individual venom components that no longer contain CCD. However, only 5 molecules are available on the market. Two recombinant wasp venom components rVes v 1 (phospholipase A1) and rVes v 5 (antigen 5) and three rApi m 1 (phospholipase A2), rApi m 2 (hyaluronidase), rApi m 10 (icarapine) for bee venom. All these venom components are species specific.

5) The basophil activation test is another diagnostic option. It is based on the expression of CD203c and CD63 molecules on the surface of activated basophils, which allows subsequent quantification using flow cytometry. The sensitivity for CD63, which is more widespread, is 89 %, whereas for CD203c it is 97 % (83). Based on the results of Bonadonna's study, it can be inferred that the reliability of the basophil activation test may be reduced in patients with mastocytosis or those with low levels of total IgE (84-85).

Massive bee envenoming

In addition to various serious allergic reactions to bee venom, an increasingly serious problem, especially in America, is the attack by large numbers of bees at once. The main reason for this is the invasive hybrid honey bee, which was created in 1955 by crossing Apis mellifera mellifera and Apis mellifera scuttellata in order to obtain a suitable species with a high honey yield for areas of South America where the spread of Apis mellifera mellifera had not been successful. However, in 1956, several queens of this African hybrid escaped, which started its uncontrolled spread, which was enhanced by its characteristics (shorter development cycle, higher drone production, more frequent swarming, less need for stores, greater range, high level of self-defence). The high degree of self-defence is the cause of their high aggressiveness, which consists in attacking a potential aggressor even at a relatively large distance from the nest, by a large number of bees and by chasing at a great distance (64,86). In addition,

the African hybrid bee releases a greater amount of venom when stung than the honey bee, despite having a smaller venom sac content. Interestingly, its venom also contains less melittin (87-88). Due to these characteristics, even an unprovoked confrontation with this bee becomes life-threatening – hence the name – killer bees. The importance of this problem is documented by the increasing number of reported cases of bee venom poisoning when large numbers of bees are being attacked. According to Betten, such an attack is considered to be a sting by more than 50 bees (89). The severity of such an attack is then a result of possible human hyperreactivity to bee venom and the total amount of venom received. Thus, bee poisoning may result in a local inflammatory reaction (characterized by swelling, redness, pain), an IgE-mediated allergic reaction (rash, itching, vomiting, diarrhea), anaphylactic shock, and a systemic toxic reaction. The severity of the problem is reflected in the increasing number of documented cases. In Brazil, between 2000 and 2013, the number of cases reached 77 066, of which 249 succumbed to poisoning. According to some authors, this is an elevenfold increase between 2000 and 2017 (90-91). In terms of mortality in Brazil, bees and their venom rank second only to snakebites (92). Manifestations of severe bee venom poisoning (systemic reactions) may initially include fatigue, nausea, vomiting, dizziness with subsequent development of hypertension, myocardial and hepatic damage, rhabdomyolysis, hemolysis, unconsciousness, and renal failure within 24 h to 6 days of an infestation. The laboratory picture may show leukocytosis, neutrophilia, and, depending on the organ damage, elevations in serum creatinine and urea, CK, AST, ALT, CRP, fibrinogen, proteuria, and glycosuria. (86,91,93-95). Until recently, there was no effective antidote that could be used in the treatment of massive bee attack, and treatment consisted of symptomatic therapy. This was due to melittin itself being poorly immunogenic due to its low molecular weight and high lytic activity (91). However, a specific antidote based on the equine antibody fragment (Fab')2 is now available and has already undergone initial clinical trials – Single-Arm, Multicenter Phase I/II Clinical Trial (94). Despite all the promising results, hyperimmune horse serum is burdened with possible side effects such as anaphylaxis and sikness serum. An alternative to the aforementioned horse serum is the use of monoclonal and recombinant antibody technology. In 2016 (Pessenda *et al.*, 2016), the results of a study based on phage display technology with the production of monoclonal antibody fragments against the main components of bee venom, melittin (Afribumab 1) and PLA2 (Afrimumab 2), were published, which are able to prolong the survival of animals that received double the LD50 in a 1:1:1 ratio (venom: Afribumab 1: Afribumab 2). A third option for the production of specific antibodies against bee venom is the use of IgY antibodies against egg yolk, which have the particular advantages of low production costs, animal welfare and less reactogenicity in mammals, assuming exclusion of allergy to egg components (96).

Investigated uses of bee venom compounds and their mechanism of action

The possibilities of using bee venom or its individual components are wide. Bee venom has anti-inflammatory, anti-cancer or antibacterial effects. It is therefore not surprising that there is intensive research into this natural product and its components, not only *in vitro* and *in vivo*, but also in ongoing clinical trials, examples of which are given in Table 3. Melittin is one of the most researched components of bee venom. Its spectrum of use is wide, but on the other hand, as with other bee venom ingredients, its use is limited, mainly due to its toxic and allergenic properties, as described above. One of the properties of melittin is the inhibition of inflammation. Melittin has, among other things, significant anti-inflammatory effects that can be used, in the treatment of a number of diseases. For example, transdermal administration of melittin via polymer microneedles suppresses levels of pro-inflammatory cytokines including IL-17 and TNF-alpha and increases the number of regulatory CD4 T-cells in a mouse/rat model of rheumatoid arthritis (97). Another example is then the reduction of neuroinflammation in a mouse model of amyotrophic lateral sclerosis, where the use of melittin led to a reduction in misfolding of alpha-synuclein and restoration of proteasomal activity in the brainstem and spinal cord (98). In the case of fibrosis accompanying renal disease, it may reduce extracellular matrix accumulation by inhibiting TGF-β-induced profibrotic gene (99). Melittin is also able to protect against the development of TAA-induced liver fibrosis via the NF-kB signaling pathway (100). The antibacterial effects of melittin are also very broad, including highly resistant pathogens. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of melittin against Extensively Drug Resistant (XDR) - *Acinetobacter baumannii* (8-16 μg/ml), methicillin-resistant *Staphylococcus aureus* (MRSA) (8-32 μg/ml), *Klebsiella pneumoniae* carbapenemase-producing (KPC-KP) (32 μg/ml-50 μg/ml) (101), MIC/MBC of melittin for Multidrug Resistant Bacteria (MDR) strains of *Acinetobacter baumannii* (0,25-0,5/0,25-1 mg/ml) (102). The possibility of treating protozoal infections such as *Trypanosoma cruzi* (33) or *Leishmania* (103) is also being investigated. Probably the most intensively investigated property, however, remains the exploitation of the anticancer properties of melittin, both on its own and as possible compounds and nanoparticles.

Table 3.

Clinical Diagnosis	Aplication Form	Timeline	Dose	Control Group	Results	Source
periarthritis humeroscapularis	i.m.	once per day, 15 days	0,0025-0,01 mg/kg	vit. B1+3 % Novocain	better effect of bee venom acupuncture compared to current treatment (vitamin B1 and novocaine 3 %) in terms of reducing pain, improving joint mobility and normalizing inflammatory cytokines	(115)
osteoarthritis	i.d.	twice a week, 4 weeks	0,1-1ml (0,5 ml to each side)	acupuncture	acupuncture has been found to have better therapeutic efficacy in comparison to traditional needle acupuncture	(116)
osteoarthritis	i.d.	once a week, 12 weeks	100 µg (total 1500 µg per visit)	Histamine 2,75 µg	honey bee venom treatment led to a reduction in pain and improved physical function of the affected knee.	(117)
rheumatoid arthritis	bee stings	once every other day, 8 weeks	5 to 15 bee stings	p.o. Methotrexate 10 mg/once a week and Celecoxib 0,2 mg/day	treating patients with rheumatoid arthritis with a combination of acupuncture and bee venom is safe and effective. No difference was observed between the two groups (P>0,05).	(118)
lumbar disc herniation	bee stings	two weeks	Control group therapy + apitherapy test (2 stings), if no adverse effects occur, followed by apitherapy every other day consisting of the addition of 1 acupuncture from the 5th to the 1st lumbar vertebrae with the application of one sting on each side up to a total of 10 stings.	magnetic thermal-vibration therapy (20 minutes each time, once a day, seven times a week, continuous treatment for two weeks), McKenzie therapy each step rested for one minute, once every two days, continuous treatment for two weeks	painless Lingnan apitherapy together with McKenzie therapy can significantly relieve pain and improve lumbar spine dysfunction in patients with intervertebral disc herniation in the lumbar region.	(119)
mild-to-moderate acne vulgaris	serum	twice daily, 6 weeks	0,7-0,9 g/dose	noncomparative study	treatment with serum containing purified bee venom was effective and no serious side effects or irritation occurred	(120)
atopic dermatitis	emollient with BV + silk-protein	twice daily, 4 weeks	not found	emolllient with silk-protein	Effective and safe treatment with significant improvement in EASI and VAS scores for pruritus	(121)

For example, in osteosarcoma, growth inhibition in a mouse model and *in vitro* in 148 B (human osteosarcoma) cells via induction of apoptosis through suppression of the Wnt/β-catenin signaling pathway has been described (104). Similarly, inhibition of MG63 (human osteosarcoma cell line) cell proliferation through activation of the apoptosis pathway mediated by endoplasmic reticulum (ER) stress has been demonstrated (105). Finally, the inhibition of proliferation in the U2 osteosarcoma cell line through regulation of Fas expression and induction of apoptosis has been documented (106). The effectiveness of melittin or its compounds in treatment is also being investigated: malignant melanoma, breast cancer including triple negative breast cancer, hepatocellular carcinoma, gastric cancer, pancreatic cancer, ovarian cancer, non-small cell lung cancer (NSCLC), prostate cancer, Hodgkin's lymphoma, glioma, colorectal cancer, squamous cell carcinoma, astrocytoma, glioblastmoid, bladder cancer, acute lymphoblastic leukemia, chronic myelogenous leukemia, bronchogenic carcinoma. Not only the use of melittin as the main representative of bee venom is being investigated. Other components are also being studied for their possible medical use. For example, PLA2 is being investigated for use in neurodegenerative diseases such as Parkinson's or Alzheimer's disease, rheumatoid arthritis, treatment of chronic wounds, prevention of inflammatory response in cisplatin-induced acute kidney injury or treatment of oxaliplatin-induced neuropathy, atopic dermatitis, bronchial asthma, inhibition of radiation-induced acute pneumonia etc. An important link in many of these effects is the influence of the immune response via regulatory T-cells. Finally, Vitellogenin has been shown to increase cell tolerance to oxidative stress (107), has the ability to bind to microbial surfaces and cause damage to microbial cell walls, and has the capacity to protect mammalian and insect cells from oxidative stress through direct protection of cell membranes (45).

Finding a safe way to use bee venom components

The main obstacle to the wider use of bee venom components remains safety, both in terms of the potential allergic reactions, the management of which to reduce this risk has been described above, and in terms of the inherent toxic effects of these substances.

Currently, many ways are being tested to reduce the toxicity of venom components that could be used for human medicine. These include melittin and its toxicity to eukaryotic cells. One way is to use the peptide or part of it and conjugate it with other compounds.

Soyoung Kim published a study where was a fragment of melittin, that had a significantly reduced hemolytic effect, fused with the pro-apoptotic peptide dKLA. PEGylation was performed to stabilize the new molecule. The resulting PEG-melittin-dKLA8-26 peptide showed excellent effect in the treatment of a mouse model of triple-negative breast cancer and its metastasis compared to the previously tested melittin-dKLA peptide (108).

Jamie E Rayahin tested an *in vitro* fusion protein of melittin and glutathione S-transferase that, unlike melittin alone, results in reduced peptide toxicity and preservation of anti-inflammatory properties at doses that exceed toxic concentrations of native melittin (109).

Another way is the use of nanoparticles.

Neelesh R. Soman described the use of perfluorocarbon nanoparticles that are capable of incorporating the non-specific amphipathic cytolytic peptide melittin into the outer lipid monolayer. The complex thus formed was able to deliver the cytolytic peptide melittin specifically to tumor cells in mice, either by nonspecific entrapment in the abnormal tumor vasculature or by binding to overexpressed integrins on angiogenic endothelial cells, thereby reducing tumor growth (110).

Chuan Huang published results using a hybrid cytosolic α-melittin peptide in which the N-terminus of melittin is linked to the C-terminus of an amphipathic α-helical peptide (α-peptide) via a GSG linker. The strong α-helical configuration allows α-melittin to interact with phospholipids and self-assemble into lipid nanoparticles. Such ordered nanoparticles containing α-melittin were then used in the treatment of melanoma in mice. The results showed at least a significant reduction in tumor size compared to the control group, but at the same time no side effects of treatment with this peptide were observed (111).

In 2023, Meiling Sun published work that aimed to synthesize nanoparticles that would be formed by self-assembly of fluorinated epigallocatechin-3-gallate (FEGCG), the main extract of green tea (FEGCG), and melittin. Subsequent experiments demonstrated not only reduced hemolytic activity of the molecule, but also antitumor efficacy in a nude mouse subcutaneous tumor model (Hep3B cells) (112).

Finally, in a randomized controlled double-blind study on volunteers, the use of purified BV essence (with reduced PLA2 and histamine) led to a reduction in the intensity of local allergic reactions (itching, redness) while maintaining a comparable anti-inflammatory effect (113).

Routes of administration and clinical testing of the effects of bee venom

Bee venom can be administered in a variety of ways: by cream, liniment or ointment, by injection, by a combination of injections and acupuncture points, or directly by a live bee sting. Currently, the most commonly used acupuncture is BV, which consists of injecting diluted bee venom into acupuncture points. Most studies and procedures use the latter option due to the combination of bioactivity of BV and mechanical stimulation of acupuncture (3,8).

We now have the results of clinical trials that have already been carried out in which bee venom has been used to treat a range of diseases. Some of these are listed in Table 3. These are studies in which purified bee venom has been used for treatment, rather than its components alone or bound to other compounds or used in combination with nanotechnology. In 2020, Soobin Jang published a systematic review of randomised controlled trials (RCTs)

on the treatment of diseases with bee venom. In total, 12 RCTs were conducted. Four of them dealt with Parkinson's disease, where in three cases the use of bee venom was associated with an improvement in the monitored parameter. The other three were related to adhesive capsulitis, pelvic inflammatory disease and osteoarthritis of the knee, which also showed an improvement in the observed score. Improvement was also demonstrated in two studies looking at low back pain, 1 RCT of delayed onset muscle pain and 1 RCT of temporomandibular disorder. Finally, in the treatment of polycystic ovary syndrome, there was a significant reduction in the LH/FSH ratio and a significant increase in progesterone levels. Adverse effects were also monitored in six of these 12 RCTs, with the most common being minor and transient skin reactions such as pruritus, rash and swelling. Serious adverse effects were not described, which may have been due to the small number of subjects but also to the significant proportion of subjects tested prior to study inclusion (114).

Despite the relatively small group of clinical studies on the positive effect of bee venom, its use in the commercial sphere is very high. Table 4 gives an example of several products containing bee venom used mainly in the cosmetic industry. In virtually all of these products sold on the Euro-American market, the venom is only one of many other substances that make up the product and contribute to its own effect. Despite the minimal information on the venom content of most of these products, their price is very high.

Table 4.

Product	Manufacturer	Market Location	Key ingredients
Cream with a drop of bee venom	www.pleva.cz	CZ	Bee venom, Caprylic/Capric Triglyceride, Butyrospermum Parkii Butter, Cetearyl Olivate Sorbitan Olivate
Bee Venom Night	www.rodial.com	UK	Bee venom melittin peptide, retinol, hyaluronic acid, matrixyl™ 3000
Bee Venom Face Mask	www.wildferns.co.nz	NZ	Bee Venom, Royal Jelly, Green Tea, Lavender Oil, Avocado Oil, Vitamin E, Canola Oil, Shea Butter, Menthol
Beesecret Serum	www.medexlife.eu	SVN	Almond Oil, Honey, Vitamin E, Bee Venom
Colored Cream with Bee Venom	www.apinfiore.com	ITL	Bee Venom, Avocado Oil, Pollen Extract, Mallow Extract, Helichrysum Extract, Natural Moisturizing Factor, Plant Complex based on Caesalpinia, Spinosa and Enteromorpha tablet
Apixin Bee venom Cream	www.beehealthyfarms.com	USA	Apis Venenum Purum, Arnica montana infused oil, Methyl Nicotinate, Eugenol, Mentha piperita oil, Eucalyptus globules oil, Oleum sinapis volatile and Proprietary Blend of pure essential oils
Bee Venom Capsules	www.honingland.nl	NL	0.1 mg of purified bee venom + vitamin C (Ascorbic acid)
Wonder Bee 24 H Anti-Age Face Cream	www.lrwonderindia.com	ITL	Bee Venom, Copaiba Sab, Vitamin A+C+E, Panthenol, Hyaluronic Acid

Conclusion

Bee products have been used by mankind for millennia. This is also the case for bee venom, whose use, especially in the cosmetics industry, is currently widespread. Its efficacy has been demonstrated in a number of *in vitro*/*in vivo* studies targeting a wide range of diseases from infectious, autoimmune to oncological. Despite all the hopes and positive effects that bee venom or its components represent and have been demonstrated in a number of studies, its use carries considerable risks in the form of toxic effects of many of its components and serious allergic reactions that can result in the death of the patient. The use of complex compounds with bee venom or its combination with nanoparticles appears to be the answer to this problem and, as studies have shown, could be a route to wider use of bee venom or its components in the treatment of a range of diseases. A parallel route is to thoroughly test patients who might undergo this treatment. A combination of both pathways may then provide a solution that allows the use of bee venom or its components with minimal risk of side effects.

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Conflict of Interest

The authors declare that no conflict of interest regarding the publication of this article.

Adherence to Ethical Standards

No applicable (review article).

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