

Review

An overview of the bioactive compounds, therapeutic properties and toxic effects of apitoxin



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1. Introduction

Apitoxin, also known as bee venom (BV), is produced in two specialized abdominal glands of worker bees and is used as a defence weapon of bees' colony (Choi et al., 2015; Zhang et al., 2016). In addition, this natural product has been used since the ancient times to relieve pain and to treat chronic inflammatory diseases such as rheumatoid arthritis, tendinitis, bursitis, skin conditions and even neurologic disorders (Moreno and Giralte, 2015).

This natural product is a light-yellow liquid, characterized by a bitter taste, pungent smell and a pH ranging from 4.5 to 5.5 (Eze et al., 2016; Hossen et al., 2017). Some studies have described that 88% of BV is water, yet its specific composition varies depending on bee species and season (Moreno and Giralte, 2015). Indeed, BV has been reported to contain a complex of biologically active compounds among which enzymes (phospholipase A₂ [PLA₂], lysophospholipase, hyaluronidase, acid phosphomonoesterase; α -glucosidase, dipeptidyl peptidase IV and vitellogenin), peptides (melittin [MLT], apamin, mast cell degranulating [MCD], mastocytolytic peptide, scapin, adolapin, minimine; apidaecin; tertiapin; melittin F; cadiopep; procamine A, B, pamine, and protease-inhibitors), biogenic amines (histamine, dopamine, noradrenaline, norepinephrine, neurotransmitters) and other compounds such as amino acids (γ -aminobutyric acid, α -amino acids), carbohydrates (glucose, fructose), pheromones (Iso-pentyl acetate, n-butyl acetate, iso-pentanol, n-hexyl acetate, n-octyl acetate, 2-nonanol, n-decyl acetate, benzyl acetate, benzyl alcohol and (2)-11 -eicosen-1-ol) and minerals (P, Ca and Mg) (Nguyen et al., 2015; Tusiimire et al., 2015; Rady et al., 2017; Moga et al., 2018; Lee et al., 2018). Among these compounds, MLT is the main therapeutic ingredient of BV, representing between 40 and 60% of the dry weight (Abd-Elhakim et al., 2014).

However, it is important to take into account that some BV constituents (such as PLA₂, lysophospholipase, hyaluronidase, MLT, apamin and MCD) can exert toxic effects, leading to clinical signs and

symptoms of envenomation. On the other hand, other compounds, like phosphatase and α -glucosidase, are non-toxic (Cornara et al., 2017).

BV may be obtained from the beehives using electric stunning devices. After, impurities and residues are removed and the remaining purified compound is lyophilized (Han et al., 2013a). *In nature*, bee venom may be injected into the victims through bees' sting and the amount of venom per sting ranges between 50 and 140 μ g (Moreno and Giralte, 2015). The responses of the human organism include local and limited or systemic and extensive inflammatory reactions, immune responses and anticoagulant effects (Eze et al., 2016; Cornara et al., 2017).

The most successful drugs developed by the pharmaceutical industry have been isolated from plants, microbes and marine organisms (Ratcliffe et al., 2014). However, only around 900 pharmaceutical products for human usage have been isolated from insects (Hassan et al., 2015). Regarding BV, the majority of the published works are preliminary studies developed *in vitro* and *in vivo* using animal models, mostly mice, while little evidence comes from studies performed directly to prove BV therapeutic activities in humans.

Therefore, this review aims to outline the published literature regarding the pharmacological properties and medical applications of Apitoxin and its bioactive compounds as well as to summarize some of the health concerns related to its application.

2. Bioactive compounds of bee venom and their biological effects

Recently, the investigation of bioactive compounds isolated from BV has attracted the attention of several researchers. The majority of the published reports aims to investigate the pharmacological properties of BV components using cell cultures or in animal models, while very little information comes from studies involving humans (Komi et al., 2018). Table 1 summarizes the therapeutic activities of peptides, enzymes and biogenic amines contained in Apitoxin.

Abbreviations: BV, Bee venom; CNS, Central Nervous System; COX, Cyclooxygenase; MCD, Mast-Cell-Degranulating peptide; MLT, Melittin; PLA₂, Phospholipase A₂

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Table 1
Bioactive compounds of bee venom and their implications in human health.

Bioactive compounds	Biological activities	References
MLT	Anti-cancer (solid tumours)	Li et al. (2006); Liu et al. (2016a); Ip et al. (2012); Jeong et al. (2014); Jin et al. (2018); Jo et al. (2012); Kong et al. (2016); Mahmoodzadeh et al. (2015); Moreno and Giralto (2015); Rady et al. (2017); Ratcliffe et al. (2014); Shin et al. (2013); Su et al. (2015); Zarrinnahad et al. (2018); Park et al. (2010); Lee et al. (2018); Wang et al. (2009); Wang et al. (2017); Zhang et al. (2014); Zhang et al. (2016) Zhang and Chen (2017)
	Anti-cancer (hematologic malignancies)	Saini et al. (1999); Moon et al. (2006)
	Anti-atherosclerotic	Jeong et al. (2012); Cho et al. (2013)
	Anti-arthritis	Jeong et al. (2015)
	Anti-inflammatory	Ip et al. (2012); Jeong et al. (2014); Jin et al. (2018); Jo et al. (2012); Kong et al. (2016); Leandro et al. (2015); Liu et al. (2016a); Mahmoodzadeh et al. (2015); Moreno and Giralto (2015); Rady et al. (2017); Su et al. (2015); Zarrinnahad et al. (2018)
	Antibacterial	Ip et al. (2012); Jeong et al. (2014); Jin et al. (2018); Jo et al. (2012); Kong et al. (2016); Liu et al. (2016a); Mahmoodzadeh et al. (2015); Rady et al. (2017); Su et al. (2015); Zarrinnahad et al. (2018); Leandro et al. (2015); Moga et al. (2018); Shi et al. (2016); Shin et al. (2017)
	Antiviral	Hood et al. (2013); Jallouk et al. (2014); Leandro et al. (2015); Moga et al. (2018); Wachinger et al. (1998)
	Pro-apoptotic	Yang et al. (2007); Han et al. (2014); Lee et al. (2014); Leandro et al. (2015); Kim et al. (2018); Moga et al. (2018)
	Analgesic	Ahn et al. (2016)
	Anti-fibrotic	Shin et al. (2017)
	Anti-diabetic	Hossen et al. (2017)
	Haemolysis	Tosteson et al., (1985)
	Neuro-protective	Han et al. (2014)
	Antifungal	Shin et al. (2017)
	Anti-fibrotic	Park et al. (2014)
	Anti-cancer (solid tumours)	Ratcliffe et al. (2014)
	Anti-inflammatory	Kim et al. (2017a); Shin et al. (2017)
Apamin	Anti-atherosclerotic	Kim et al. (2015)
	Antibacterial	Leandro et al. (2015)
	Suppresses biliary fibrosis	Kim et al. (2017b)
	Antibacterial	Boutrín et al. (2008); Leandro et al. (2015)
PLA ₂	Anti-arthritis	Eze et al. (2016)
	Anti-trypanosomiasis	Boutrín et al. (2008)
	Neuroprotective	Jeong et al. (2011)
	Anti-malarial	Deregnaucoirt and Schrével (2000)
Secapin	Anti-cancer	Ratcliffe et al. (2014); Wang et al. (2009)
	Anti-HIV	Fenard et al. (2001); Ratcliffe et al. (2014)
	Antifungal	Lee et al. (2016a)
	Antibacterial	
Adolapin	Anti-elastolytic	
	Anti-fibrinolytic	
	Anti-inflammatory	dos Santos-Pinto et al. (2018); Moga et al. (2018); Nitecka-Buchta et al. (2014); Komi et al. (2018)
	Anti-nociceptive	
Apidaecin	Anti-pyretic	
	Antibacterial	Li et al. (2006a); Van Vaerenbergh et al., (2013)
Protease inhibitor	Antibacterial	Eze et al. (2016)
	Anti-inflammatory	
	Haemostasis	
	Inward-rectifier K ⁺ channel block	Cornara et al. (2017)
Tertiapin	Beta adrenergic blocker, anti-arrhythmic	Moreno and Giralto (2015); Vick et al. (1974)
	Mast cell degranulation, histamine release	Moga et al. (2018)
	Anti-inflammatory	Eze et al. (2016); Moreno and Giralto (2015)
	Allergic response and Hypersensitivity	dos Santos-Pinto et al. (2018)
	Enhance the noxious action of substances	Reitinger et al. (2008)
	Use in BV immunotherapy	Hossen et al. (2016)
	Embryogenesis; angiogenesis, diffusion of toxins and drugs, metastasis	Bordon et al. (2015)
	Allergic response	Eze et al. (2016)
Histamine	Local and systemic inflammation	

2.1. Peptide components

2.1.1. Melittin

Melittin (MLT), also known as allergen Api m4 (Hossen et al., 2017), is the main compound of BV, comprising approximately 40–60% of its dry weight. MLT (together with apamin) has been reported to be found only in bee venom produced by the genus *Apis* (Moreno and Giralto, 2015). It is a linear, amphipathic, cationic and α -helical polypeptide molecule composed of 26 amino acid residues and is soluble in water (Silva et al., 2015; Kachel et al., 2018).

Recent studies provided evidence on several biological properties of MLT (Table 1). MLT has been reported to be a very potent anti-

inflammatory agent, being 100 times more potent than hydro-cortisol in animal models (Vick and Shipman, 1972; Eze et al., 2016). The anti-inflammatory activity of this compound has been explored in several settings, including acne vulgaris, neuro inflammation, atherosclerosis, arthritis and liver inflammation (Lee and Bae, 2016).

Its ability to disrupt lipid cell membranes and antimicrobial properties has also attracted great interest, especially for the treatment of drug-resistant infections (Choi et al., 2015; Cornara et al., 2017; Nguyen et al., 2015; Uddin et al., 2016). These authors also observed that MLT significantly inhibits the replication of both enveloped [melittin molecules fuses with viral envelope, forming pore-like attack complexes] and non-enveloped viruses, decreasing their infectivity

through surface charge interaction.

This compound is also cytotoxic against cancer cells among other mechanisms through the activation of apoptotic pathways (both caspase-dependent and caspase-independent) and PLA₂ pathways (Lee et al., 2018; Jin et al., 2018; Shin et al., 2017; Zhang and Chen, 2017; Wang et al., 2017). Indeed, the growth inhibitory potential and the capacity to suppress tumour metastasis of MLT has been observed in several types of cancer cells like liver, renal, prostate, breast, lung, bladder, gastric and leukemia, suggesting that it may be a potent agent to use in chemotherapy (Liu et al., 2016; Moreno and Giral, 2015; Kong et al., 2016).

Melittin has also been shown to induce neural plastic changes in pain-signalling pathways by sensitization and activation of nociceptor cells (Wehbe et al., 2019).

A schematic representation of the action mechanisms of some biological effects of MLT (anticancer, anti-inflammatory, antimicrobial and anti-nociceptive) are presented in Fig. 1.

In spite of the promising properties that are attracting much interest from pharmacological and biotechnological fields, MLT (whose initial structure is helical, when embedded in a lipid bilayer) can change to a dimeric form that destabilizes and alters artificial lipid bilayers containing dioleoylphosphatidylcholine (Somwongin et al., 2018). Therefore, MLT may induce morphologic changes in a dose-dependent manner, being a nonselective cytolytic peptide that may induce the lysis of red blood cells and human peripheral blood lymphocytes, among others (Gajski et al., 2016). Indeed, while in small doses MLT has anti-inflammatory effects, increases capillary permeability, in higher doses it may cause inflammation, itching, irritation and local pain (Lee and Bae, 2016). According to the literature, this compound may cause slight, moderate and severe irritation at 0.5 mg/ml, 1 mg/ml and concentrations above 2 mg/ml, respectively (Somwongin et al., 2018). The mechanism underlying toxicity is based in the disruption of phospholipid bilayers which lead to mast cells' lysis and the release of compounds like lysosomal enzymes, histamine and serotonin, triggering pain and inflammation. Therefore, MLT together with hyaluronidase

and PLA₂, are responsible for venom allergenic properties breaking up membranes cells and enhancing their cytotoxic effect (Kachel et al., 2018).

Attempts have been made to avoid these side effects via phosphorylation of MLT in specific amino acid residues; the peptide phosphorylated in ¹⁸Ser elicited lower allergenic responses when compared to native melittin. In addition, ongoing studies are assessing MLT incorporation into nanoparticles in order to decrease its the nonspecific lytic activity (Abd El-Wahed et al., 2018).

2.1.2. Apamin

Apamin is a neurotoxin with 18 amino acid residues that comprises 2–3% of BV dry weight (Rady et al., 2017; Moga et al., 2018). As mentioned previously, apamin and MLT are “specie specific” compounds whose presence has only been reported in the BV of the *Apis* genus (Moreno and Giral, 2015). Apamin is permeable to the blood–brain barrier, affecting the central nervous system (CNS) and producing hyperactivity and convulsions. It is well known that this peptide is as a specific and high-affinity blocker of small-conductance Ca²⁺-activated K⁺ channels (SK-channels) (Kim et al., 2017a; Zhang et al., 2018). Peripherally, apamin selectively and potently affects the potassium permeability of muscle cells membranes and, when in high doses, may cause muscle spasms and respiratory failure. This characteristic has been widely explored and apamin has been used in the literature to identify the activity of the SK-channels in different cells such as visceral smooth muscle, microglial cells, adrenal cortex, hepatocytes and ventricle cells (Voos et al., 2017). In spite of this, recent studies demonstrated that this compound is also capable of inhibiting the channel Kv1.3 with high affinity (Voos et al., 2017). These two types of channel are frequently co-expressed in different tissues among which immune system cells (T cells, macrophages and dendritic cells).

Several researchers studied health-related applications of Apamin (Table 1) as anti-inflammatory (Shin et al., 2017), antibacterial, anti-fungal, anti-fibrotic (Shin et al., 2017), anti-cancer (Ratcliffe et al., 2014), anti-atherosclerosis (Kim et al., 2015), anti-nociceptive and

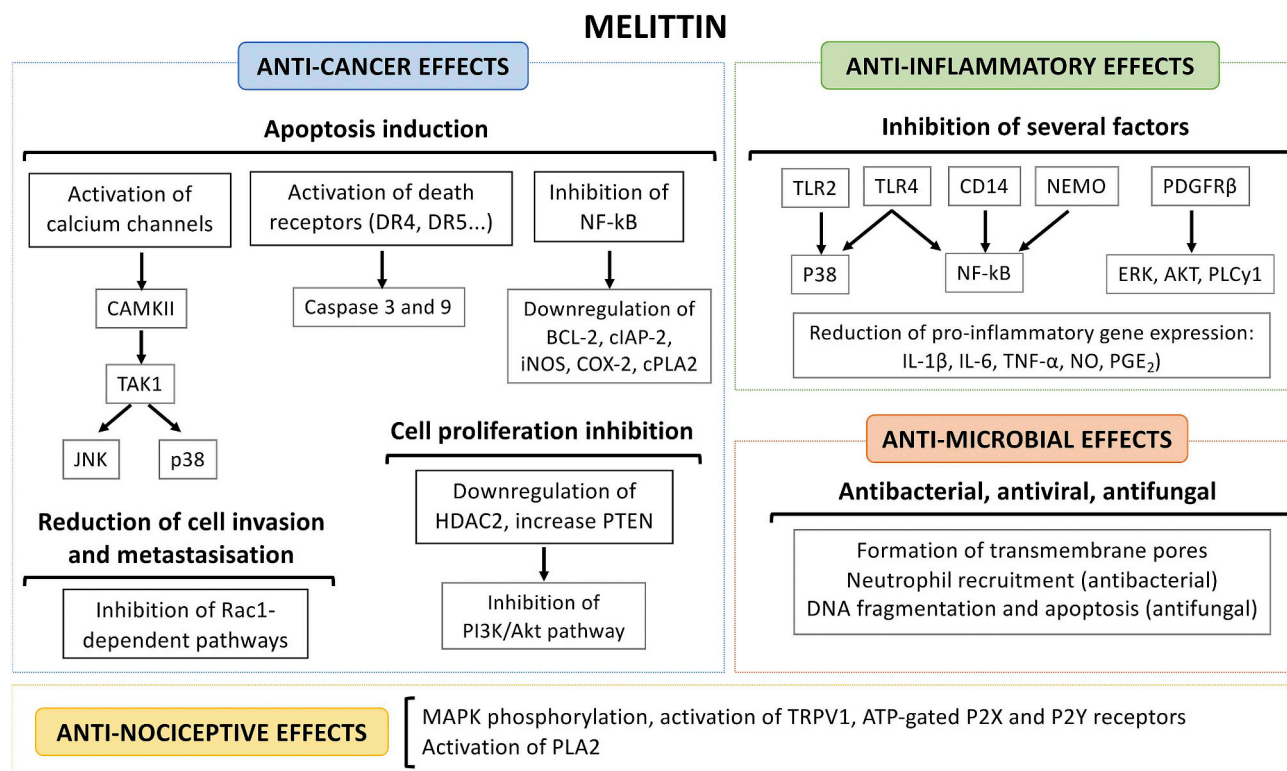


Fig. 1. Schematic representation of the action mechanisms of some biological effects of melittin.

cytotoxic against cancer cells (Son et al., 2007). It has also been reported to protect undamaged neurons but also to be able to restore the function of silent ones (Moreno and Giral, 2015). In mice models with cholestatic liver disease apamin suppressed the deposition of collagen and the expression of fibrogenic genes (Kim et al., 2017b), suggesting its role has a potential therapeutic target.

2.1.3. Mast-cell-degranulating peptide

Mast cell degranulating (MCD), also known as peptide 401, (Cherniack and Govorushko, 2018), is another important compound with 22 amino acids and two disulphide bridges which comprises around 3% of BV dry weight. It promotes mast-cells degranulation, triggering inflammatory reactions (Rady et al., 2017; Komi et al., 2018). MCD has unique immunologic properties, causing histamine release at concentrations of less than 0.1 mg/mL while acting as an anti-inflammatory compound at higher concentrations (Moga et al., 2018). Indeed, MCD has been recognized as a potent anti-inflammatory agent (Moreno and Giral, 2015). This compound is also a neurotoxin by blocking fast-inactivating and slow-inactivating calcium-activated potassium channels (KC-channels), therefore increasing neuronal excitability (Cornara et al., 2017). However, the effects in the CNS are poorly studied (Chen et al., 2016).

2.1.4. Secapin

Secapin, comprising only 0.5–2% of BV dry weight, is a potent neurotoxin containing 25 amino acid residues and a disulphide bridge between Cys 9 and Cys 20 (Rady et al., 2017). It is a serine protease inhibitor-like peptide with anti-fibrinolytic (plasmin inhibitor), anti-elastolytic, anti-fungal and antibacterial activities; nevertheless, the mechanisms underlying such actions are poorly understood (Lee et al., 2016).

2.1.5. Adolapin

Adolapin is a basic polypeptide with 103 amino acid residues, representing 0.5–1% of BV dry weight matter (Rady et al., 2017). This compound has been reported to be anti-inflammatory, anti-nociceptive and anti-pyretic, mainly due to the inhibition of cyclooxygenase (COX), PLA₂ and lipoxygenase, thereby, prostaglandin synthetase system, following a biphasic dose-response relationship (Moga et al., 2018; Nitecka-Buchta et al., 2014; Komi et al., 2018).

2.1.6. Apidaecin

Apidaecin-type peptides are a series of small proline-rich peptides containing 18 to 20 amino acid residues (Li et al., 2006a). This non-helical and heat-stable compound is active against a wide range of human pathogens and plant-associated bacteria, through a bacteriostatic process (Van Vaerenbergh et al., 2013). These compounds are the largest group of proline-rich antimicrobial peptides known to date and appear to be non-toxic to human and animal cells, making them potential candidates for the development of new antimicrobials in the future (Li et al., 2006a).

2.1.7. Protease inhibitor

The protease-inhibitor comprises 0.1–0.8% of BV dry weight and has been reported to have anti-inflammatory and anti-fibrinolytic effects (Eze et al., 2016). Other studies revealed anti-microbial activity against fungi, gram-positive and gram-negative bacteria (Yang et al., 2017).

2.1.8. Tertiapin

Tertiapin comprises < 0.1% of BV dry weight and is a small presynaptic peptide with 21 amino acids that blocks inward rectifier KC channels expressed in heart, epithelial cells and CNS (dos Santos-Pinto et al., 2018; Rady et al., 2017). Despite being classified as a neurotoxin (as occurs for MCD and secapin), the information on its pharmacological and toxicological effects in the CNS is quite limited (Chen et al.,

2016). Nowadays, tertiapin is used only for K⁺ channel modulation; in the future it is expected to be useful for treating disorders in atrio-ventricular transmission (Cornara et al., 2017).

2.1.9. Cardiopep

Cardiopep is a peptide comprising less than 0.7% of BV dry matter (Moreno and Giral, 2015) reported to possess beta adrenergic and anti-arrhythmic effects (Vick et al., 1974).

2.1.10. Other peptides

Other minor constituents of BV are pamine (1–3% of dry weight matter), minimine (2%), procamine A, B (1–2%), and melittin F, a fragment of MLT in which the first seven residues from the N terminus are missing (< 0.1%) (Chen et al., 2016; Rady et al., 2017).

2.2. Proteins

2.2.1. Phospholipase A₂ (EC 3.1.1.4)

Phospholipase A₂ has been reported to be the most abundant enzyme of BV, corresponding to 10–12% of its dry weight matter (dos Santos-Pinto et al., 2018). Due to its high molecular weight and strong antigenicity, this enzyme has been pointed as the major cause of the systemic allergic reactions to BV (Gajski et al., 2016; Hosseini et al., 2016). On the other hand, several recent reports unveiled the potentiality of PLA₂ in the treatment of human disorders (Table 1) including Parkinson's disease, asthma, several types of cancer, as well as immune mediated disorders like cisplatin-induced nephrotoxicity, hepatotoxicity and Lupus nephritis (Ratcliffe et al., 2014). Its role in bacteria and parasite infections was also described. For instance, Boutrín et al. (2008) observed that PLA₂ inhibited Gram-negative bacteria and the parasitic protozoan *Trypanosoma brucei*. This study also confirmed the antibacterial activity against *Escherichia coli*, *Enterobacter cloacae* and *Citrobacter freundii* (Boutrín et al., 2008), *Burkholderia pseudomallei*, *Staphylococcus aureus*, *Streptococcus salivarius* and *Lactobacillus casei*.

2.2.2. Hyaluronidase (EC 3.2.1.35)

Hyaluronidase is widely distributed in nature, particularly in animal venoms such as bee, scorpion, wasp, spider, fish and crustaceans (Bordon et al., 2015). This enzyme is composed by 350 amino acids and specifically degrades hyaluronic acid in the extracellular matrix of skin matrix, allowing the penetration of venom components deeply into the blood stream (Marković-Housley et al., 2000). Other roles of hyaluronidase in envenoming process are cell membrane disruption, pore formation, mast cell degranulation (dos Santos-Pinto et al., 2018), dilatation and increased permeability of blood vessels (Hosseini et al., 2016). This enzyme is the second major allergen of BV (following Phospholipase A₂), comprising 1–2% of dry weight matter (Cornara et al., 2017). Even though hyaluronidase has little toxicity it can enhance the effect of other toxins present in venoms, contributing to the local and systemic effects of envenoming (Bordon et al., 2015).

Regarding the application of hyaluronidases for human health, these may act as an adjuvant to enhance the absorption and dispersion of injected drugs, be used in ophthalmologic surgeries and in the treatment of diseases associated with excessive “ground substance”, either applied subcutaneously or in a topical way (Reitinger et al., 2008). These enzymes may also play a role in various physiological mechanisms like angiogenesis, embryogenesis, metastasis formation, cicatrization and in several inflammatory settings like pneumonia, meningitis and sepsis (Bordon et al., 2015).

2.2.3. Acid phosphomonoesterase (EC 3.1.3.2)

The acid phosphomonoesterase, also known as acid phosphatase, is a glycoprotein and potent allergen found in BV, representing approximately 1–2% of its dry weight matter (Rady et al., 2017). This compound has been reported as a stronger elicitor of histamine release from basophils of sensitized humans. However, the pathophysiological role

of this enzyme following BV inoculation remains largely unexplored. Even so, some reports exist regarding the potential for immunotherapy against BV (Hossen et al., 2016).

2.2.4. Dipeptidyl peptidase IV

Dipeptidyl peptidase IV (Api m 5) is present in BV in low concentrations (< 1% of dry weight) and has been identified as one of the proteins of higher molecular weight (102 kDa). It appears to be responsible for the cross-reactivity between bees and wasps' venoms (Antolín-Américo et al., 2017).

This proteolytic enzyme cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position, what leads to the activation of BV components and promotes hypersensitivity reactions (dos Santos-Pinto et al., 2018).

2.2.5. Vitellogenin

Vitellogenin is another allergen found in BV, present in larger amounts in the BV secreted by honeybee queens. This enzyme is believed to be involved in several processes such as hormone signalling, food-related behaviour, stress resistance, immunity and longevity (dos Santos-Pinto et al., 2018), yet the underlying mechanisms remain unclear (Blank et al., 2013). Its role in the hypersensitive reactions has also been reported (dos Santos-Pinto et al., 2018).

2.2.6. Other proteins

Several proteins are present in BV in low concentrations, comprising < 1% of its dry weight matter: Lysophospholipase (EC 3.1.1.5) also known as phospholipase B (Hossen et al., 2016; Rady et al., 2017) and α -D-Glucosidase (EC 3.2.1.20) (Hossen et al., 2016; Rady et al., 2017). To date, their biological activities are poorly documented.

2.3. Biogenic amines

2.3.1. Histamine

This small molecule comprises 0.5–2% of BV dry weight matter and is the major component among the category of biogenic amines (Moreno and Giral, 2015). During bee venom allergy, histamine is the principal inflammatory mediator released immediately in the skin, mainly mediated by mast cell degranulation peptide. This amine promotes capillary leakage, causes itching, swelling and pain (Rady et al., 2017).

2.3.2. Dopamine and noradrenaline

Dopamine and noradrenaline are also low molecular weight compounds found in BV comprising < 1% of its dry weight matter (Rady et al., 2017). These compounds are ionotropic agents and have been pointed out to be protease inhibitors, playing a role in haemostasis and acting as anti-inflammatory agent (Nitecka-Buchta et al., 2014).

2.4. Other components

BV also contains amino-acids (like γ -aminobutyric acid and α -amino acids), carbohydrates and pheromones (iso-pentyl acetate, n-butyl acetate, iso-pentanol, n-hexyl acetate, n-octyl acetate, 2-nonanol, n-decyl acetate, benzyl acetate, benzyl (Rady et al., 2017)).

3. Therapeutic applications of bee venom in human health

The use of bee products (honey, pollen, propolis, bees' wax, royal jelly and bee venom) to treat some human illnesses is known as apitherapy and dates back from thousands of years, with their healing properties being cited in many religious texts (Cornara et al., 2017). BV has been used since around 3000 BC in ancient Egypt and Greece (Yang et al., 2017; Lee et al., 2018). Throughout the decades the use of apitherapy expanded from the Eastern to the Western countries, being currently practiced all over the world (El-Wahab and Eita, 2015),

mostly as a complement to the conventional medicine practices. Notwithstanding, the understanding of the chemical composition and actions of BV dates back only 50 years ago (Moreno and Giral, 2015).

Depending on the health condition under treatment BV can be used topically (by applying a cream, liniment, or ointment), via injection, either through acupuncture or directly through a live bee sting. Currently, the most commonly used method is BV acupuncture, which consists in the injection of diluted bee venom into acupuncture points (Lee et al., 2015).

In the last decade, as result of increasing investigation worldwide, the usefulness of BV has been reported for several diseases and pathologic contexts (Table 2), in studies performed *in vitro* and *in vivo*, in animal models or enrolling humans. Among other settings, the effects of BV were demonstrated in multiple sclerosis and Parkinson's disease (Ostrovsky and Ehrlich, 2018), dementia (Silva et al., 2015), atherosclerosis (Lee and Bae, 2016), osteoarthritis (Lee et al., 2012), human immunodeficiency virus (HIV) (Jallouk et al., 2014), periodontitis (Gu et al., 2019), Diabetes mellitus (Hossen et al., 2017) and rheumatoid arthritis (Son et al., 2007; Brito et al., 2018). The effects of BV and its isolated bioactive compounds have also been described in several types of solid cancer like breast, ovarian, bladder, lung, liver and prostate, as well as on hematologic malignancies (Moga et al., 2018). Other therapeutic activities described are antibacterial, anti-fibrosis and antinociceptive (Cho et al., 2018; Cornara et al., 2017). One of the best documented effects of BV is its powerful anti-inflammatory activity, which is particularly due to the presence of melittin. Indeed, even though BV may produce local inflammation when delivered in high doses, when used in controlled concentrations it has potent anti-inflammatory effects by influencing endogenous cortisol production and suppressing inflammatory mediators among which tumour necrosis factor alpha (TNF- α) and interleukin 1- β (Lee and Bae, 2016). This property is very relevant having into account the important role of chronic inflammation in the pathogenesis of a wide range of conditions like cardiovascular diseases, obesity and diabetes, asthma, autoimmune disorders, inflammatory bowel disease and neurological diseases.

In addition, due to its anti-ageing properties, BV has been increasingly more used in the manufacture of cosmetics products (Choi et al., 2015). All of these biological activities are related to the presence of some bioactive compounds mainly the MLT, PLA₂ apamin, secapin, tertiapin and MCD (Zarrinnahad et al., 2018).

In this context, this natural product and its isolated components are valuable resources to be used in the future for the design and development of drugs. However, besides the fact that the BV concentration that allows maximising the beneficial effects while decreasing the adverse ones remains unknown, many of the active compounds are far from being explored (Uddin et al., 2016). In addition, the toxicity of BV remains a big dilemma for researchers and clinicians; indeed, while small compounds and peptides mainly mediate local reactions causing discomfort, larger proteins are involved in direct tissue damage or in systemic hypersensitive reactions including anaphylaxis (Jakob et al., 2017). Thereby, yet much information is available regarding the potential applications of BV, further studies must be developed to clarify the optimum dose, its adverse effects (particularly on risk groups like pregnant and breast-feeding women) and the possible interactions with conventional drugs. A graphical overview of current strengths, weaknesses, opportunities and threats of bee venom is depicted in Fig. 2.

4. Applications of bee venom in the cosmetic industry

BV has several promising properties that aroused the interest of the dermatocosmetic industry (Komi et al., 2018). Indeed, besides its antimicrobial, antibacterial and anti-inflammatory properties, some studies reported the potentiality of BV to reduce skin aging manifestations such as skin tone changes, wrinkles, sagging and evenness (Choi et al., 2015). Notwithstanding, the potential to cause skin irritation could be a major concern (Somwongin et al., 2018).

Table 2
Pharmacological activities and medical applications of *Apitoxin*.

Effects of bee venom	References
Amyotrophic lateral sclerosis	Lee and Bae (2016) [<i>in vivo</i> , animal models]
Anti-ageing	Choi et al. (2015) [<i>in vivo</i> , humans; <i>in vitro</i>]; Han et al. (2013a) [<i>in vivo</i> , humans; <i>in vitro</i>]; Tusiimire et al. (2015) [<i>in vitro</i>]; Han et al. (2015) [<i>in vivo</i> , humans; <i>in vitro</i>]; Lee et al. (2015) [<i>in vitro</i>]
Anti-arthritis	Lee et al. (2012); Wang et al. (2002); Jeong et al. (2015); Son et al. (2007) [all studies performed <i>in vivo</i> , in humans];
Anti-dandruff	Prakash and Bhargava (2014) [<i>in vitro</i>]
Anti-fibrotic	Lee et al. (2015) [<i>in vivo</i> , animal models]
Anti-inflammatory	Sobral et al. (2016); Kim et al. (2016); Mohammadi et al. (2015) [all performed <i>in vitro</i>]
Anti-nociceptive	Cornara et al. (2017); Son et al. (2007) [both <i>in vitro</i>]
Anti-atherosclerotic	Moreno and Giralto (2015); Lee and Bae (2016) [both <i>in vitro</i>]
Anticancer (hematologic malignancies)	Safaeinejad et al. (2013) [<i>in vitro</i>]; Mohseni-Kouchesfahani et al. (2017); Moga et al. (2018) [all performed <i>in vitro</i>]
Anticancer (solid tumours)	Son et al. (2007); Liu et al. (2016); Park et al. (2011); Cornara et al. (2017); Ip et al. (2008); Jo et al. (2012); Kim et al. (2015a); Oršolić (2009); Choi et al. (2014); Gajski et al. (2016); Tu et al. (2008); Lee et al. (2015b); Wang et al. (2015); Sisakht et al. (2017); Jung et al. (2018); Moga et al. (2018) [all performed <i>in vitro</i>]
Antidiabetic	Prakash and Bhargava (2014) [<i>in vivo</i> , humans]; Hossen et al. (2017) [<i>in vivo</i> , mice and rabbits];
Antimicrobial	Han et al. (2016); Lee (2016); Hegazi et al. (2017); Al-Safar et al. (2018); Park et al. (2018) [all performed <i>in vitro</i>]
Antimutagenic	Varanda et al. (1999); Cornara et al. (2017) [all performed <i>in vitro</i>]
Antioxidant	Sobral et al. (2016); Somwongin et al. (2018) [all performed <i>in vitro</i>]
Antiproliferative and cytotoxic	Gülmez et al. (2017); Sobral et al. (2016) [all performed <i>in vitro</i>]
Antiviral	Hassan et al. (2015); Hood et al. (2013); Kim et al. (2015a); Wachinger et al. (1998); Jallouk et al. (2014) [all performed <i>in vitro</i>]
Apoptosis	Moon et al. (2006); Jang et al. (2003); Park et al. (2014); Ip et al. (2008); Doo et al. (2012); Choi et al. (2014); Sisakht et al. (2017) [all performed <i>in vitro</i>]
Alleviating post-stroke shoulder pain	Cho et al. (2013a) [<i>in vivo</i> , humans]
Chemotherapy-induced peripheral neuropathy	Park et al. (2012); Yoon et al. (2012) [<i>in vivo</i> , humans]
Chronic osteoarticular pain	Shin et al. (2012); Seo et al. (2017) [<i>in vivo</i> , humans]
Complex regional pain syndrome	Kim et al. (2014) [<i>in vivo</i> , humans]
Depression	El-Wahab et al. (2015); Cherniack and Govorushko (2018) [<i>in vivo</i> , humans]
Lateral epicondylitis of the elbow	Jung et al. (2014) [<i>in vivo</i> , humans]
Neuro-protective	Doo et al. (2012); Jung et al. (2015); Hwang et al., 2015 [all performed <i>in vitro</i>]
Parkinson's disease	Cho et al. (2018); Hwang et al., 2015; Ostrovsky and Ehrlich (2018); Kim and Jeon (2014); Lee and Bae (2016) [<i>in vivo</i> , humans]
Periodontitis	Kim et al. (2018); Gu et al. (2019) [both <i>in vitro</i>]
Postherpetic neuralgia	Lee et al. (2014a) [<i>in vivo</i> , humans]
Radio-protective	Cornara et al. (2017); Gajski et al. (2016) [<i>in vitro</i>]
Rheumatoid arthritis	Son et al. (2007); Lee et al. (2003); Lee et al. (2003a); Brito et al. (2018) [all performed <i>in vivo</i> , using animal models]
Sexual dysfunction	Lee and Yu (2014) [<i>in vivo</i> , humans]
Skin disease (acne vulgaris)	Han et al. (2013a); Han et al. (2016a); Kim et al. (2015) [<i>in vitro</i> , in humans];

APITOXIN

Molecular formula: $C_{129}H_{224}N_{38}O_{31}$

Global composition

88% water; the remaining 22% follow the distribution:

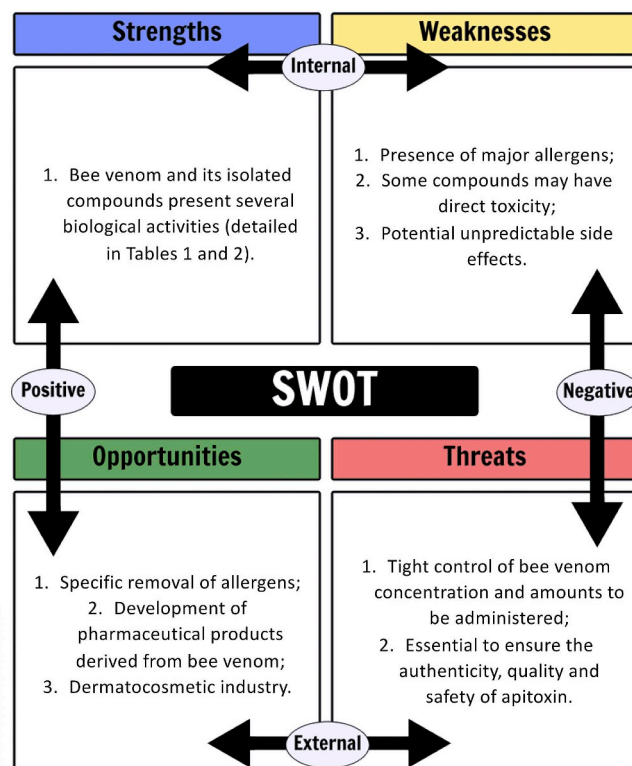
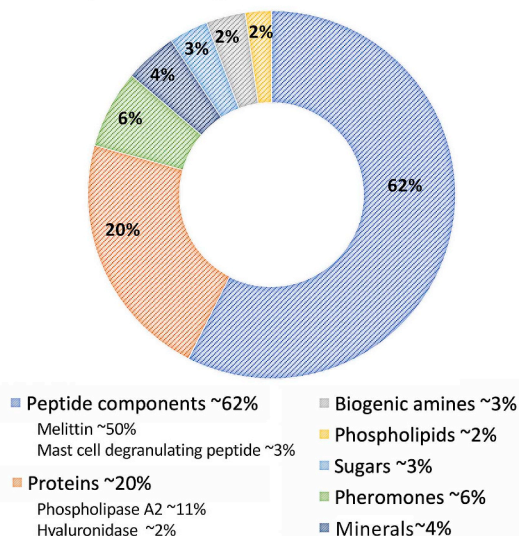


Fig. 2. Graphical overview of apitoxin composition, strengths, weaknesses, opportunities and threats.

Han et al. (2013a) observed that cosmetics containing purified BV had a better efficiency in reducing microorganisms' concentration and the number of skin lesion, suggesting that purified BV could be a good candidate for developing new formulations for acne vulgaris treatment. Later, Han et al. (2015) made it possible to objectify BV efficacy in terms of reducing wrinkle count, area, and depth in subjects with photodamaged skin. Also, these authors stated that the irritation potential is negligible and that long-term treatment with bee venom-containing cosmetics was safe.

Current, the majority of the cosmetic products available on the market do not mention exactly the content of BV, being marketed as containing "purified bee venom" or "bee venom extracts" without further specification. Efforts must be done in order to invest in products' standardization and labelling, in order to overcome this very important limitation. Indeed, a method for the detection and quantification of melittin, as a marker of BV concentration, was developed by Tusiimire et al. (2015).

5. Bee venom: safety and practical considerations

During the bee sting a small amount of BV is injected into the victim (maximum of 0.3 mg per sting). The severe allergic reactions, which among insects are most frequently induced by the order hymenoptera, are more often related to IgE-mediated reactions induced by allergens rather than to the direct toxic activity of other venom components (dos Santos-Pinto et al., 2018). To date, the main allergens found in BV are phospholipase A2 (Api m1), hyaluronidase (Api m2), acid phosphomonoesterase (Api m3), melittin (Api m4), dipeptidyl dipeptidase IV (Api m5), protease inhibitor (Api m6), CUB serine protease 1 (Api m7), carboxylesterase (Api m8), serine carboxypeptidase (Api m9), icarapin (Api m10), major royal jelly protein 9 (Api m 11.0101 & Api m 11.0201) and vitellogenin (Api m12) (Kołaczek et al., 2017). According to the International Union of Immunological Societies (IUIS), Phospholipase A₂, hyaluronidase and apamin are the major allergens contained in BV (Tusiimire et al., 2015; Park et al., 2013; Lee et al., 2018). These compounds induce changes in cell membranes, degrade hyaluronic acid, enhance toxins' spreading into tissues and play a role in the destruction of extracellular matrix, among other mechanisms (Reitinger et al., 2008).

According to the literature, more than 50% of the general population has IgE reactivity to the major allergens of BV which can cause a spectrum of allergic reactions, which usually appear within 10 min (Vazquez-Revuelta and Madrigal-Burgaleta, 2018). The reactions induced by BV affect the skin but also the gastrointestinal, respiratory and cardiovascular systems, ranging from mild, local swelling to systemic and severe reactions that in some cases can even be life-threatening (Marković-Housley et al., 2000).

Even though it has been reported that venom immunotherapy is effective in the majority of Hymenoptera venom allergic patients, early and late side effects to the injections have been found in 20–40% of the patients (Blank et al., 2013). The most common early side effect is the acute local reaction, followed by weakness and dyspnoea; rhinitis and abdominal pain are more common as late side effects (Kołaczek et al., 2017).

In order to ensure patients' safety and to take advantage of the potentialities of BV while reducing the side effects, some researchers suggest the removal of the allergens PLA₂, hyaluronidase, acid phosphomonoesterase and apamin (Brito et al., 2018). In that context, Lee et al. (2018) developed an effective strategy for MLT purification from BV which also effectively removed PLA₂ and apamin. Also, Park et al. (2013) found that the use of 50% ethanol aqueous solution for the purification and analysis of BV, the content of apamin and other allergens was significantly decreased, yet MLT was kept stable. In addition, Lee et al. (2015c) and Brito et al. (2018), by means of ultrafiltration using 10 kDa molecular weight cut-off filters, made it possible to effectively remove PLA₂.

Even though BV has promising healing properties it may cause several adverse events, whose occurrence is mostly unpredictable. Local inflammatory but also systemic reactions, among which erythema, pruritus, swelling, pain, anaphylaxis, neurological complications among other nonspecific reactions may occur (Yang et al., 2017). Therefore, the researchers and health care practitioners must be aware and pay careful attention to the safety rules regarding the BV concentration and amounts to be administered in the patients (Zhang et al., 2018).

The beehive products have been used in the human therapeutic activities since memorable times. Concretely, the practices of acupuncture using the venom have been extensively used in Oriental medicine even though their effectiveness and safety profile is still controversial due to the limited evidence (Zhang et al., 2018).

In our days, the toxicity of BV it is a big dilemma for the researchers. Several authors reported that BV is safe for humans, allowing to take advantage of its antimicrobial (Uddin et al., 2016), anti-inflammatory, anti-wrinkle (Lee et al., 2015c), and anti-acne properties (Han et al., 2016a), among others. The topical application of BV is advantageous to the residual harmful systemic side effects (Nitecka-Buchta et al., 2014). On the other hand, several other studies raised concerns on BV toxicity (Lee et al., 2015c; Kołaczek et al., 2017). A meta-analysis performed in 2015, including 145 studies, revealed that the median frequency of patients experiencing adverse events associated with BV immunotherapy was 29% (in the audit studies); and BV acupuncture had a 261% increased relative risk for the occurrence of adverse events, in comparison with normal saline injection (Park et al., 2015). Systemic reactions occurred in 14% of the patients, half of which were severe. This study concluded that adverse events related to bee venom therapy are frequent, suggesting that safety is still a limitation to take into consideration.

Gülmez et al. (2017) reported that BV is highly toxic to cancer cells but also to non-tumorigenic cells, suggesting that the use of BV in the treatment of malignant tumours should be re-evaluated due to the undesirable cytotoxicity; further studies must be performed to clarify this issue.

Recently, a case reported raised a major issue. A Spanish women of 55 years age, without clinical records of any disorder, risk factors or previous reactions of any kind to Hymenoptera, had been attending apitherapy sessions for two years with good tolerance. One day, immediately after a live bee sting the women suddenly developed dyspnoea, wheezing and loss of consciousness and, due to multiorgan failure, died a few weeks later (Vazquez-Revuelta and Madrigal-Burgaleta, 2018). According to the researchers this case is the first report of death associated to the BV therapy due to complications of severe anaphylaxis.

A recent *in vivo* study performed in 2018, using mice, reported that the approximate median lethal dose (LD₅₀) of the BV is 13.19 mg/kg (Lashein et al., 2018). The amount of BV released during a bee sting ranges between 50 and 140 µg (Moreno and Giral, 2015), much lower than the average lethal dose. However, as previously described, it may be sufficient to trigger an anaphylactic reaction. Therefore, for each therapeutic use the optimal dose and application method must be determined, in order to ensure that the beneficial effects are not hampered by harmful adverse reactions.

Last but not the least, it is essential to ensure the safety and quality of the BV: before use, a battery of physicochemical and microbiological analysis must be performed. Attention must be paid also on the storage conditions: BV may be stored up to 12 months at room temperature or up to 24 months if kept in a cool place (2–8 °C), in the original container, unopened, in a dry place and away from strong light (Lee et al., 2015a).

6. Conclusions

Bee venom has been used since the ancient times in the treatment of different disorders. On the last decades, several studies provided

scientific evidence regarding the wide range of biological activities of Apitoxin. In spite of that, the adverse effects that not uncommonly follow BV administration are still a challenge for researchers and healthcare practitioners worldwide. Indeed, even though BV immunotherapy appears to be efficacious and safe (when used in controlled conditions), side effects related to BV therapy cannot be underscored. In addition, the generation of clinical-grade and sterile pharmaceutical products derived from BV is still challenging, mostly related to identification, isolation, and purification of bioactive components; further developments are expected. In the future, practitioners should be trained in order to optimize the BV concentrations that allow maximising the clinical beneficial effects while avoiding adverse ones; further studies (mainly randomized controlled trials) are required.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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