# Animal venoms: therapeutic tools for tackling Parkinson's disease

**Henrique de Oliveira Amaral, Victoria Monge-Fuentes, Andréia Biolchi Mayer, Gabriel Avohay Alves Campos, Kamila Soares Lopes, Luana C. Camargo, Matheus Ferroni Schwartz, Priscilla Galante and Márcia R. Mortari**

Laboratory of Neuropharmacology, Department of Physiological Sciences, Institute of Biological Sciences, University of Brasilia, Brasilia, Brazil

**Parkinson's disease (PD) is a neurodegenerative pathology of the central nervous system, mainly involving the selective and progressive loss of dopaminergic neurons from the substantia nigra, resulting in motor and non-motor symptoms. PD remains an incurable ailment; thus, treatments are limited to symptom alleviation. With long-term use, conventional treatments can become inefficient, often triggering possible side effects. Considering these drawbacks, drug discovery constantly turns to nature as a source of efficient therapeutics. Thus, this review explores animal venoms as a rich source of bioactive compounds with potent neuropharmacological profiles for the development of effective adjuvant treatments with fewer side effects, ultimately aiming for the neuroprotection of dopaminergic neurons and the symptomatic relief of PD.**

## Introduction

Parkinson's disease (PD) is a neurodegenerative and progressive disorder that affects the central nervous system (CNS) of 2–3% of the population over the age of 65 years. PD symptomatology is commonly associated with cardinal motor complications, such as bradykinesia, postural instability, resting tremor and rigidity. Additionally, PD is accompanied by several non-motor symptoms, bringing patients to overall disability [1]. Classical neuropathological hallmarks that are linked to PD include the selective and progressive loss of dopaminergic neurons from the substantia nigra (SN) and the histopathological presence of Lewy bodies, composed mainly by protein aggregates of α-synuclein [2]. Furthermore, available knowledge on PD lacks a well-defined etiopathogenic mechanism; thus, several hypotheses have linked genetic factors, environmental toxins, neuroinflammation, mitochondrial disturbances, excitotoxicity, protein misfolding and aggregation, impairment of protein clearance pathways, cell-autonomous mechanisms and advanced age [3,4] as possible causes.

*Corresponding author:* Monge-Fuentes, V. (victoriananobio@gmail.com)

Despite the efforts made through arduous research, PD remains an incurable ailment and available pharmacological treatments are mainly focused on treating classical motor symptoms and improving patient quality of life [5]. Moreover, current treatment is unable to slow or halt the progression of the disease and, with long-term use, some of these drugs can become inefficient and/or even trigger adverse effects [6]. In view of these limitations, drug discovery repeatedly turns to nature as a source of more-efficient therapeutics. Thus, herein, we assess animal-venom-derived compounds as promising lead candidates for the development of pharmacological probes and drugs able to translate as effective adjuvant treatments with fewer side effects, ultimately aiming for the neuroprotection of dopaminergic neurons and the symptomatic relief of PD [7].

## Animal venoms as a promising adjuvant treatment for Parkinson's disease

Animal venoms translate as the result from millions of years of evolutionary pressure, which led to the development of several venom-derived compounds with binding specificity, high affinity and ample potency profiles [8]. Considering that the extraordinary




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repertoire of animal venoms evolved as predation and defense tools, they often act on the nervous and cardiovascular systems with the ability to disrupt a range of vital physiological and biochemical processes [9]. Such a disruptive neurological effect can be attributed to the ability that many of these venom-derived compounds have to modulate synapses by acting with remarkable potency and selectivity on various biological targets, including receptors, ion channels and enzymes [10].

Given that animal venoms exhibit chemical diversity of bioactive substances [inorganic salts, small organic molecules (<1 kDa), polypeptides (2–9 kDa) and high-molecular-weight molecules (>10 kDa) such as proteins and enzymes], several studies have investigated their mode of action on targets, exploring the biochemical and genomic composition of the compounds, and potential to generate new pharmaceuticals. In addition, with the ever-increasing advances in the development of new analytical tools (i.e., mass spectrometry, transcriptomics and proteomics screenings), the use of venom components as therapeutics represents a growing and promising approach [11]. Most neurological disorders are currently treated with symptomatic-only drugs, which can often be associated with a range of adverse peripheral effects. Therefore, the selectivity seen in a variety of venom-derived components obtained from bees, scorpions, snakes and lizards could potentially lead to the development of new pharmacological probes and drugs with efficiency and fewer side effects for various diseases, including PD [7], as we shall examine in detail below.

## Bee venom

Bee venom (BV) is a complex fluid comprising a variety of components, including peptides (mellitin, apamin, adolapin), enzymes (phospholipase $A_2$, hyaluronidase, acid phosphomonoesterase, lysophospholipase and -D-glucosidase) and lower-molecular-weight compounds (biogenic amines and histamine), secreted from the gland of honey bee, *Apis mellifera,* exhibiting a wide range of pharmacological activities [12,13]. BV has been considered a possible medicine for several conditions, such as neurological and neurodegenerative disorders, which includes PD [13–16] (Table 1). In view of this, several experimental studies have elucidated its possible activity as a neuroinflammatory modulator and neuroprotector. Research performed with lipopolysaccharide (LPS)-stimulated BV2 microglial cells showed that BV inhibited the production of nitric oxide (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, prostaglandin E2 (PGE2), nuclear factor (NF)-κB and proinflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6] [17–19]. In addition, BV increased the proportion of CD4*CD25*Fosp3 regulatory T cells (Tregs) and it also reduced microglia activation in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced (MPTP) mouse model for PD [20]. Further, the neuroprotective effect of BV was described as it reduced astrocyte activation in a subchronic MPTP-induced model, attenuating dopaminergic neuronal loss [21].

Moreover, BV inhibits apoptosis, as noted by Jung *et al*. who evaluated its neuroprotective effect on NSC34 cells after rotenone treatment. It was further observed that BV decreased caspase-3 expression and blocked the JNK and ERK1/2 signaling pathways [22]. Additionally, the neuroprotective mechanisms of BV decreased caspase-3 activity and DNA fragmentation, suppressing proapoptotic factor Bax production and increasing the expression of antiapoptotic factor Bcl-2 in SH-SY5Y neuroblastoma cells [23]. BV also exhibited effects against glutamate excitotoxicity [24] and, in a 6-hydroxidopamine (6-OHDA) rat model for PD, it diminished contralateral forelimb akinesia and improved the function of cortico-basal ganglia circuits [25].

Interestingly, although the therapeutic landscape of BV for the treatment of parkinsonism in preclinical studies seems vast and promising, unfortunately it does not mirror results obtained in clinical studies developed in this field, where few trials have been registered (NCT01341431 and NCT01970813) (https://clinicaltrials.gov/; information on clinical trials was obtained with the search criteria: 'melittin and Parkinson's disease'; 'apamin and Parkinson's disease'; 'bee venom and Parkinson's disease'; 'snake venom and Parkinson's disease'; 'Exendin-4 and Parkinson's disease'; 'exenatide and Parkinson's disease'). Summarizing the available clinical evidence, first, the efficacy of repeated subcutaneous injections of BV on PD motor symptoms and disease progression was investigated [26]. However, owing to the lack of clear symptomatic or disease-modifying effects compared with placebo, future studies were suggested using higher BV individual doses and administration frequency. Following this pilot study, consequent studies treated patients with BV acupuncture (BVA) or acupuncture, used as adjuvant therapy, reporting that acupuncture and BVA groups showed significant improvement in several motor tests; thus, effectiveness remained uncertain as it could be attributed either to the pharmacological actions of the venom or to the mechanical effect induced by the acupuncture stimulation [14]. Another study developed by the same research group assessed the combination of BVA with manual acupuncture, revealing that the combined treatment was safe with significant motor function improvement [27]. Later, a triple-armed study reported a prominent placebo effect; however, long-term effects on symptom improvement were detected for the active treatment group (BVA and acupuncture); suggesting that BV seems to have an actual therapeutic biological effect [28]. As further noted by Awad *et al*., BV could be a promising adjuvant treatment for PD; however, there is still lack of enough evidence to validate the efficacy of BV and/or its components for PD treatment in a clinical setting [16]. In our point of view, trial design for BV investigation needs to be improved, using larger sample sizes, higher doses and frequency, additional robust control groups covering all possible variables, considering interactions when combined (or not) with PD conventional treatments. Because BV contains a series of compounds with different pharmacological effects and the increasing importance given to the research and development of new strategies to tackle neurological disorders, we will further explore the main components of BV and assess their potential effect for PD treatment as reported, to our knowledge, so far only in preclinical studies.

### *Melittin*

Among the main components of BV we highlight melittin – an alkaline and amphiphilic peptide comprising 26 amino acid residues [16]. Melittin makes up 40–60% of the total of dry BV and it is recognized as one of the compounds with more pharmacological activities. Thereby, recent studies are attempting to elucidate the mechanism of action of melittin and actual effect for the treat-

**TABLE 1**

**Pharmacological effects studied in whole bee venom (*Apis mellifera*) and its main components for the treatment of Parkinson's disease.**

| Venom or compound | Experimental model | Treatment dose | Outcome | Refs |
|---|---|---|---|---|
| BV | LPS-stimulated BV2 microglial cells | 1, 10, 100 ng/ml | Potential anti-inflammatory effect with reduction of TNF-α, iNOS and NO production in activated microglia in dose-dependent manner | [17] |
| BV and melittin | LPS-stimulated BV2 microglial cells | 0.5, 1, 2 μg/ml | Suppression of LPS-induced inflammation by reducing NO, PGE2 and cytokines (IL-1β, IL-6 and TNF-α) production.<br>Both compounds regulated different pathways (JNK and Akt pathways and NF-κB activation via regulation of the IκBα pathway) attenuating NO production | [18] |
| BV | LPS-stimulated BV2 microglial cells | 0.625, 1.25, 2.5 μg/ml | Suppression of NO production and proinflammatory cytokines expression (TNF-α and IL-6) in a dose-dependent manner. Inhibited NF-κB transcriptional activity in MyD88-dependent manner | [19] |
| BV | MPTP-induced PD model | 1 mg/kg | Increased Treg infiltration *in vivo* and reduced microglia activation. Treg infiltration in brain can induce neuroprotection effect | [20] |
| BV | Subchronic MPTP-induced PD model | 200 μg/kg | Attenuated astrocyte activation in SN in subchronic MPTP-induced PD model, preventing dopaminergic neurons from death | [21] |
| BV | Rotenone-induced cell death in motor neuron line NSC34 | 2.5 μg/ml | Neuroprotective effect by blocking the JNK and ERK1/2 signaling pathways<br>Suppressed rotenone-induced activation of caspase-3 and mitochondria impairment | [22] |
| BV | SH-SY5Y human neuroblastoma cell | 1, 10 and 100 ng/ml | Reduced expression of Bax and increased expression of Bcl-2 and caspase-3 activation<br>Attenuated PI3K/Akt-mediated signaling pathway | [23] |
| BV | N2a neuroblastoma cell/BV2 murine microglial cell | 2.5 and 5 μg/ml | Inhibited phosphorylation of ERK, p38 and JNK in N2a neuroblastoma cells and JNK phosphorylation in BV2 microglial cells<br>Antiapoptotic effect and protected cells from glutamate-induced toxicity | [24] |
| BV and apamin | 6-OHDA-induced PD model/haloperidol-induced catalepsy model | BV (1 and 3 μg/kg)<br>Apamin (0.1, 0.2 or 0.4 mg/kg) | Reversed catalepsy behavior induced by haloperidol<br>Modulated the basal ganglia signaling by counteracting imbalanced activity of the trans-striatal pathways. Results implicate in an efficacy of BV on motor PD symptoms, which could be exerted by apamin | [25] |
| BV | Randomized, double-blind, placebo-controlled, parallel-group single-center trial | 100 μg (Alyostal®) (in 1 ml of NaCl 0.9%) | BV group did not show clear symptomatic or disease-modifying effects when compared to placebo group | [26] |
| BV | Randomized controlled clinical trial | 0.1 ml diluted to 0.005% in distilled water | Patients undergoing BVA and acupuncture as adjuvant treatment twice a week for 8 weeks improved motor symptoms, as evaluated by UPDRS score<br>No significant difference was observed between treatment groups (acupuncture and BVA) | [14] |
| BV | Prospective open-label clinical study | 0.1 ml diluted to 0.005% in normal saline | Patients undergoing BVA and acupuncture as adjuvant treatment twice a week for 12 weeks improved motor symptoms, as evaluated by UPDRS score | [27] |
| BV | Double-blind, randomized controlled clinical trial | 0.05 mg/ml | BVA and acupuncture improved motor symptoms when administered twice a week for 12 weeks as an adjuvant treatment<br>Prominent placebo effect | [28] |
| Melittin | ALS mouse model (hSOD1$^{G93A}$ mice) | 0.1 μg/g | Improvement of motor behavior in the ALS mouse model and mitigation of microglial activity, reducing TNF-α expression<br>Attenuated post-translational modification of □-synuclein, which could be related to the restoration of proteasome activity, as seen in brainstem and spinal cord of the transgenic mice | [30] |
| Melittin | $H_2O_2$-induced neurotoxicity SH-SY5Y human neuroblastoma cell | 0.5, 1 and 2 μg/ml | Suppressed expression and activation of caspase-3 and modulated expression of the Bcl-2 protein family (Bax and Bcl-2) | [31] |

**TABLE 1 (*Continued*)**

| Venom or compound | Experimental model | Treatment dose | Outcome | Refs |
|---|---|---|---|---|
| PLA2 | MPTP-induced PD model | 0.5 mg/kg | Attenuated microglia activation and diminished $CD4^+$ T cell infiltration in the brain of mouse with parkinsonism<br>Affinity for mannose receptor (CD206) on dendritic cells, promoting Treg induction in CD4 T cells | [41] |
| PLA2 | A53 T α-Syn transgenic mouse model of ALS<br>(A53 T α-Syn transgenic mice) | 0.2 and 1 mg/kg | Increased microglia M2 phenotype, changing the M1: M2 ratio, promoting an anti-inflammatory response<br>Improved motor behavior and reduced α-Synuclein in the spinal cord of transgenic mice | [42] |
| $PLA_2$ and melittin | MPTP-induced PD model | Extracts with diverse composition of $PLA_2$ and melittin | $PLA_2$ improved motor behavior and protected loss of dopaminergic neurons. Melittin did not exert the same effects<br>$PLA_2$ induced Treg cell differentiation and decreased $CD4^+$ T cells with Th1 and Th17 phenotypes. Melittin did not exert the same effects | [43] |
| Apamin | 6-OHDA-induced PD model | Bilateral injections of apamin (0.05 or 0.1 ng per side, dissolved in a volume of 0.5 ml, in 0.9% sterile NaCl) | Improvement of akinetic deficits produced by nigrostriatal dopaminergic lesions when injected directly into the subthalamic nucleus of parkinsonian rats | [47] |
| Apamin | 6-OHDA-induced PD model | 0.1 or 0.3 mg/kg | Alleviation of non-motor symptoms induced by 6-OHDA, reducing anhedonia and anxiety, preserving short-term social and spatial memories<br>Reduced motor symptoms, playing an important part in cellular mechanisms of emotional behaviors and spatial memory mediated in the basal ganglia | [50] |
| Apamin and BV | MPTP-induced PD model | BV (12 mg/kg/BW and 120 mg/kg/BW) and apamin (0.5 mg/kg/BW, 1.0 mg/kg/BW and 1.0 mg/kg/BW) | Protection of dopaminergic neurons from SNpc against death in a chronic parkinsonian model<br>Apamin did not preserve dopaminergic nerve terminals, suggesting that another molecule in BV might enhance apamin activity | [51] |

Abbreviations: 6-OHDA: 6-hydroxidopamine; ALS: amyotrophic lateral sclerosis; Akt: protein kinase B; BV: bee venom; BVA: bee venom acupuncture; BW: body weight; ERK extracellular signal-regulated kinase; IL: interleukin; iNOS: inducible nitric oxide synthase; JNK: C-jun N-terminal kinase; LPS: lipopolysaccharides; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO: nitric oxide; PD: Parkinson's disease; PGE2: prostaglandin E2; $PLA_2$: phospholipase A2; SN: substantia nigra; SNpc: substantia nigra pars compacta, TNF: tumor necrosis factor; Treg: regulatory T cell; UPDRS: Unified Parkinson's Disease Rating Scale.

ment of neurodegenerative diseases (Table 1). Moon *et al*. reported that melittin reduced neuroinflammation (decreased IL-1β, IL-6, TNF-α and NO production, and iNOS, PGE2 and COX-2 expression) in a LPS-stimulated BV2 microglial model [18]. Other experimental studies have also contributed to the understanding of these mechanisms [29,30].

Considering the strategies that might be effective for the treatment of PD, melittin has been shown to reduce neuroinflammation (TNF-α expression reduction), decrease -synuclein misfolding and restore proteasomal activity in the brainstem and spinal cord in an amyotrophic lateral sclerosis model (hSOD1G93A transgenic mice) [30]. Moreover, melittin exhibited antiapoptotic effects by increasing antiapoptotic factor Bcl-2 production while inhibiting proapoptotic factor Bax and caspase-3 synthesis in a model of neuroblastoma (cell line SH-SY5Y) [31]. In addition, the pharmacological effects of melittin and BV with emphasis on dopaminergic related behavior were evaluated, showing that both compounds caused catalepsy and reduced motor stereotypies induced by apomorphine in rodents. Melittin further exhibited antipsychotic properties, without inducing side effects, suggesting an interaction of BV and melittin with the dopaminergic system [32] (Fig. 1).

Overall, at the preclinical level, melittin shows several desired characteristics that depict it as an interesting candidate to tackle some PD neuropathogenic mechanisms. Nevertheless, considering issues on selectivity, cell membrane morphological alterations and possible cytotoxic activity [33,34], experiments in animal models need to be more conclusive to elucidate the effects and properties of melittin with emphasis on the route of administration, possible side effects as well as target cells and/or organs before considering their application in clinical trials.

### *Phospholipase $A_2$*

Phospholipase $A_2$ ($PLA_2$) is a catalytic enzyme that breaks down phospholipids into phosphate and fatty acids [35]. Interestingly, $PLA_2$ has been isolated from the venom of a number of animals, such as snakes, spiders, lizards and bees ($bvPLA_2$) [36]. Besides its enzymatic activity, $bvPLA_2$ has the ability to bind to the CD206 receptor in dendritic cells [37].

The adaptive immune response has an important role in PD, and recent studies demonstrated that infiltrating T cells were surrounded by dopaminergic neurons, attenuating neuronal death [38]. By contrast, Tregs are known for protecting neurons from degeneration [39,40]. In this sense, studies have reported that $PLA_2$ increases the Treg population and decreases neuronal death in the MPTP model [41] and the -synuclein transgenic model [42]. When $PLA_2$ binds to CD206 in dendritic cells, PGE2 is activated, inducing T-cell differentiation into T-cell regulation via Foxp3 expression [41]. Moreover, treatment with $PLA_2$ changes the M1:M2 ratio by decreasing the M1 phenotype of microglia and increasing the M2

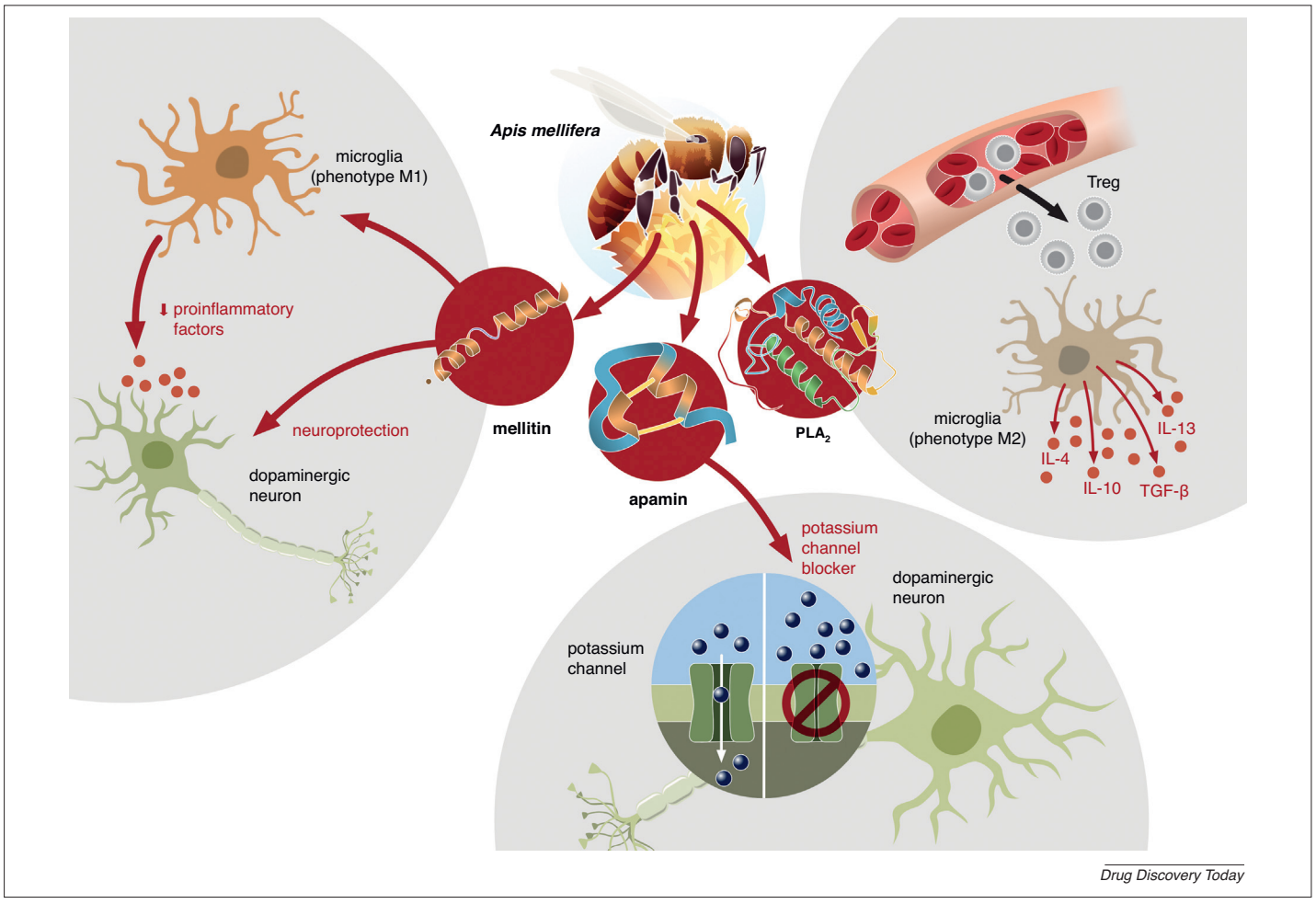


**FIGURE 1**

Mechanisms of action for the main components found in bee venom (BV) (*Apis mellifera*) on the central nervous system. **Left:** Mellitin presents a suppressive effect over inflammatory responses in M1 microglia by reducing the release of proinflammatory factors [decreases production of interleukin (IL)-1β, IL-6, tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO), and expression of nitric oxide synthase (iNOS), prostaglandin E2 (PGE2) and cyclooxigenase 2 (COX-2)]. Moreover, mellitin shows a neuroprotective effect by inducing an antiapoptotic effect [increases B-cell lymphoma protein 2 (Bcl-2) and inhibits Bcl-2-like protein 4 (Bax)]. **Bottom:** Apamin binds to small conductance Ca2+-activated K+ channels (SK-channels), selectively blocking their activation, serving as a dopaminergic neuron regulator. **Right:** phospholipase $A_2$ ($PLA_2$) increases Treg cell population at the central nervous system, causing a neuroprotective effect. $PLA_2$ also increases M2 microglia, producing an anti-inflammatory response.

phenotype, which produces an anti-inflammatory response (Fig. 1). Besides, in the -synuclein transgenic model, $PLA_2$ decreases the -synuclein inclusions in the spinal cord and mice treated with $PLA_2$ improve their performance in the Pole locomotion test; however, the mechanism by which PLA2 acts on -synuclein is still unclear [42].

In addition, another study compared the effects of $bvPLA_2$ and melittin (combined or not) extracted directly from BV in an MPTP-induced mouse model of PD. Kim et al. observed that $bvPLA_2$ exerted a potent neuroprotective effect, with improved motor function. Moreover, $bvPLA_2$ inhibited proinflammatory T cell phenotypes and induced Treg cell differentiation in a dose-dependent manner. Melittin alone exhibited a limited therapeutic effect on motor behavior and neuroprotection. However, when a melittin (15%) and $PLA_2$ (78%) combined extract was applied, a considerable neuroprotective effect with motor function improvement was seen. As discussed by the authors, melittin could have a synergistic effect, contributing to PLA2 therapeutic activity [43] (Table 1). Although bvPLA2 might possess a neuroprotective anti-inflammatory effect, its mechanism of action still needs to be elucidated.

$bvPLA_2$ presents several attractive characteristics for PD treatment; nevertheless, it also interacts with cell membranes by inducing the breakage of phospholipid bilayers, triggering damage in cell and organelle membranes, implying a nonselective effect of this enzyme [36]. Considering the variety of targets where $PLA_2$ could interact, preclinical experiments need to work with a critical approach concerning nonselectivity issues and possible $PLA_2$ unwanted effects and properties before entering clinical development.

### *Apamin*

Apamin stands as another important component isolated from BV with relevant activity in PD models (Table 1). Apamin is a peptide composed of 18 amino acid residues and the ability to cross the blood–brain barrier (BBB) [44,45]. Furthermore, apamin binds to

small-conductance $Ca^{2+}$-activated $K^+$ channels, also known as SK channels (composed of either SK2 or SK3 subunits), selectively blocking their activation [46] (Fig. 1). In the context of PD treatment, the selectivity of apamin to bind to either subunit is relevant considering that SK channels regulate dopaminergic neurons and are found in high density in the basal ganglia [47]. SK channels are also responsible for maintaining neuronal excitability after hyperpolarization; thus, channel blockade by apamin facilitates neuronal firing [48,49]. Therefore, when 6-OHDA-lesioned animals were treated with apamin, motor and non-motor symptoms were counteracted and extracellular dopamine concentration in the striatum was increased [50]. Likewise, when apamin was directly injected into the CNS in a 6-OHDA animal model, akynetic symptoms were reduced [47]. In an MPTP model, apamin protected dopaminergic cell body neurons in SN pars compacta (SNpc); however, dopaminergic neuronal terminals were not spared [51]. Electrophysiological studies have further demonstrated that when afterpolarization is blocked by apamin in dopaminergic neurons, an increase in the number and frequency of action potentials is observed [48]. These changes in firing patterns decrease the interaction between *N*-methyl-D-aspartate (NMDA) receptors and SK channels, contributing to an increase in dopamine release [52].

## Scorpion venom

Scorpion venoms are composed of a variety of different chemical molecules, including inorganic salts, free amino acids, peptides, heterocyclic components, proteins (mainly enzymes) and other substances that are still unknown [53,54]. Scorpion venoms as well as the animal's organs have been used since medieval times to cure several pathologies [55]. Indeed, peptides isolated from scorpion venoms exhibit multiple pharmacological effects, including analgesic, anticancer, cardiovascular and immunosuppressive actions [56].

Aiming to elucidate whether any of these components present useful activity for PD treatment, some studies have analyzed their direct or indirect interaction with ion-channel function mechanisms. It is notable that venom-derived peptides obtained from scorpions exert their effect mainly by the blockage of $Na_v$ channels [57]. In this sense, it has been demonstrated in an MPTP non-human primate model of PD that motor cortex stimulation by high-frequency electrical interference modulates the subthalamo-pallido-cortical loop, alleviating parkinsonian symptoms [58]. This type of stimulus is more specifically related to the blockage of $Na^+$ and $Ca^{2+}$ voltage-gated currents, leading to the interruption of spontaneous activities in subthalamic neurons [53]. In this context, Cn2 toxin, purified from the venom of Mexican scorpion *Centruroides noxius*, induces a voltage-sensor-trapping mechanism in the $Na^+$ channel, isoform $Na_V1.6$ current, as recorded in wild-type cerebellar Purkinje cells, blocking firing with selectivity and binding stability at the channel site [59]. Thus, Cn2 scorpion toxin represents a promising pharmaceutical candidate for further studies based on PD motor symptom relief.

Molecules derived from venoms can also be applied for the construction of ligand-modified targeting delivery systems [60]. Such is the case of a 36-amino-acid peptide originally isolated from *Leiurus quinquestriatus* scorpion venom: chlorotoxin (ClTx) [61]. Considering the various obstacles to effectively delivering L-Dopa to the CNS for PD therapy, Xiang and co-workers applied nanobiotechnological strategies to create stealth liposomes modified with ClTx (ClTx-LS) to serve as L-Dopa carriers in an MPTP-induced PD model in mice. This enabled a system that reverses coordinated movement impairments caused by MPTP through successful delivery of L-Dopa across the BBB to the brain microvascular endothelial cells, consequently increasing dopaminergic levels in the SN and striatum [60].

In view of the capacity of compounds to work as neuroprotectors, a heat-resistant peptide, named SVHRP, was isolated from the venom of Chinese scorpion *Mesobuthus martensii* (previously *Buthus marthensii* Karsch). SVHRP protects dopaminergic neurons in the SN, improving behavioral deficits in parkinsonian rats and mice [62]. In a subsequent study, additional beneficial actions were reported for SVHRP when tested in an early-stage 6-OHDA-PD rat model. SVHRP significantly reversed the effects caused by neurotoxin 6-OHDA, acting as a neuroprotector that prevents biochemical (reversed abnormal activities of monoamine oxidase-B, superoxide dismutase and malondialdehyde in the mitochondria of neurons in the midbrain) and ultrastructural damage (improved optical density of dopaminergic neurons) in midbrain neurons [63] (Table 2).

## Snake venom

Originally, snake venoms were used exclusively for antivenom production; however, as their components were isolated and biological activity tested, application was extended for diagnostic tests, biotechnological tools and drug development, becoming the main source of venom-derived drugs [11,64,65]. Snake venoms involve a complex mixture of peptides, proteins and small molecules that present an effect particularly in the CNS, cardiovascular, muscular and vascular systems [64,66]. Additionally, minor components in snake venoms might be accountable for potential therapeutic use as antiparasitic, antitumor, neuro and ischemic tissue protection [65].

As an approach to identify novel therapeutic activities derived from snake venoms, the use of C-Map (Connectivity Map composed of a database of gene expression patterns) has been proposed as a biological activity screening tool. Through the use of C-Map, venom of the South American pit viper *Bothrops jararaca*, incubated with MCF7 cells, was associated to 19 drugs used for the treatment of neuropsychiatric disorders, including hits for two antiparkinsonian drugs: metixene and lisuride. The antiparkinsonian activity seen in *B. jararaca* might be related to the presence of neurotoxins with affinity for muscarinic receptors [67]. In this respect, snake neurotoxins (polypeptides MT1 and MT2) that bind to muscarinic acetylcholine receptors (mAChRs) were isolated from green mamba venom [68]. The selectivity observed in these polypeptides is of great interest for the treatment of neurodegenerative diseases, such as PD, considering that the selective blocking of these muscarinic receptors might be of great aid for restoring normal movement [68,69].

Trophic factors have also been suggested as important therapeutics for the treatment of neurodegenerative conditions. Among their roles, trophic factors are in charge of supporting and protecting cellular subpopulations [70]. Studies have also specifically reported that trophic factors act on dopaminergic neurons *in vitro* and *in vivo*, protecting them or restoring their function after the acute effects of neurotoxins; thus, turning them into potential

**TABLE 2**

**Pharmacological effects investigated in scorpion and snake venoms for the treatment of Parkinson's disease**

| Animal | Venom or compound | Experiment model | Treatment dose | Outcome | Refs |
|---|---|---|---|---|---|
| *Leiurusquin questriatus* | Chlorotoxin (ClTx) | MPTP-induced PD mouse model | 20 mg/kg of levodopa (L-Dopa) | Liposome modified with ClTx actively delivers L-Dopa to the brain, increasing dopaminergic levels in the SN and striatum<br>Improved motor behavior in MPTP parkinsonism mouse model | [60] |
| *Mesobuthus martensii* | Scorpion venom heat-resistant peptide (SVHRP) | 6-OHDA-induced early PD rat model | 0.05 mg/kg | Protected dopaminergic neurons from death<br>Attenuated mitochondrial damage and reversed abnormal activities of MAO-B, SOD and MDA, supporting antioxidant activity of the compound | [63] |
| *Bothrops atrox* | Tripeptide isolated from the venom | Rat pheochromocytoma cell line (PC12) treated with dopaminergic neurotoxin MPP+ | 350 μg/ml | Decreased activity of proteases caspase-3 and caspase-9<br>Neuroprotection mechanism might be related with neurotrophic effect | [72] |

Abbreviations: 6-OHDA: 6-hydroxidopamine; MAO-B: monoamine oxidase; MDA: malondialdehyde; MPP+: 1-methyl-4-phenylpyridinium; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $PLA_2$: SOD: superoxide dismutase; SN: substantia nigra.

therapeutic candidates for PD [70,71]. Brain-derived neurotrophic factor (BDNF), a key growth factor, acts as a neuroprotective and pro-neuroplasticity agent, improving the therapeutic outlook of patients with neuronal pathological degeneration [62]. With this in mind, and with the aim of investigating the neuroprotective function and neurotrophic ability of a tripeptide (Glu-Val-Trp) isolated from *Bothrops atrox* venom, Martins and co-workers used PC12 cells treated with dopaminergic neurotoxin MPPT. Results showed an increase in cell viability and proliferation, as well as a decrease in apoptotic protease caspase-3 and caspase-9, both activated in PD. Additionally, the tripeptide tested on the PC12 cells treated with the dopaminergic toxins and the nontreated PC12 cells increased neuritogenesis and protected against differentiation impairment [72].

Taken together, these studies support the notion that this tripeptide offers a promising mechanism of neuroprotection against dopaminergic cell death, useful as a platform for the design of novel pharmacological agents for PD. Moreover, because the composition of *B. atrox* and *B. jararaca* venoms are related [73], the presence of this tripeptide found in *B. atrox* should be further investigated in the venom of *B. jararaca* to understand its role as a neuroprotector agent (Table 2).

## Lizard venom

Venom of *Heloderma suspectum*, known as the Gila monster, originally isolated from its saliva and now synthesized on a large scale, has been of particular interest for drug design and the pharmaceutical industry, particularly since the development of licensed drugs for the treatment of type 2 diabetes mellitus [74,75]. Some of the components identified in *Heloderma* venom include cysteine-rich secretory protein, exendin, helofensin, kallikrein, natriuretic peptide and $PLA_2$ [76]. Initial studies demonstrated that the crude venom of *H. suspectum* induced amylase release from dispersed acini from guinea pig pancreas, greatly increasing intracellular cyclic adenosine monophosphate (cAMP) [77]. Those findings motivated the search for secretory components in the venom, leading to the discovery of a 39-amino-acid peptide, named Exendin-4. This peptide did not stimulate amylase secretion, but increased intracellular cAMP [78], an effect later associated with the binding of Exendin-4 to glucagon-like peptide-1 receptor (GLP-1R) [79]. These receptors are expressed and distributed in pancreatic β cells and within the brain, in particular in the midbrain and striatum, and their activation on neurons leads to important neurotrophic and neuroprotective effects, such as neurogenesis, neuroinflammation reduction, oxidative stress protection, apoptosis inhibition and synaptic plasticity enhancement [80].

In this sense, several studies were first conducted in cellular and animal models [81–85] (Table 3), evidencing neuroprotection after administration of Exendin-4. Considering that Exendin-4 safety has already been established and as a result of the growing evidence of its efficacy in animal models, a proof-of-concept single-blind study with 45 moderate PD patients was conducted, demonstrating that twice-daily injections of Exendin-4 resulted in improvement in the Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) after 12 months of treatment [86]. In addition, the effect was maintained after a 12-month washout period [87], indicating a possible neuroprotective effect. A second, double-blind, placebo-controlled trial was performed in 62 patients treated with weekly injections of Exendin-4 for 48 weeks, followed by a 12-week washout period, revealing improved motor skills in the Exendin-4-treated group compared with placebo [88]. Nevertheless, considering that Exendin-4 is immediately available once it is injected subcutaneously and its elimination half-life is 2.4 h [89], Chen *et al.* worked on a formulation, named PT302, that encapsulated Exendin-4 in biodegradable poly lactic-co-glycolic acid (PLGA) microspheres, providing sustained release in a 6-OHDA hemiparkinsonism rat model. Pre- and post-treatment with PT302 significantly reduced methamphetamine-induced rotation after lesion and post-treatment significantly increased tyrosine hydroxylase in the lesioned SN and striatum, suggesting that PT302 has a neuroprotective effect for nigrostriatal dopaminergic neurons at a clinically relevant dose [90]. Collectively, these results indicate relevant potential of Exendin-4 and other GLP-1 receptor agonists in the treatment of PD (Table 3).

**TABLE 3**

**Pharmacological effects researched in Gila monster lizard (*Heloderma suspectum*) venom for the treatment of Parkinson's disease**

| Venom or compound | Experimental model | Treatment dose | Outcome | Refs |
|---|---|---|---|---|
| Exendin-4 | *In vitro* [anterior wall of the lateral ventricle of 5-week-old mice (C57 black)]<br>*In vivo* (6-OHDA-induced PD model) | *In vitro* (100 nM)<br>*In vivo* (0.1 μg/kg) | Neurogenesis modulator effect as it induced proliferation and differentiation of *in vitro* and *in vivo* neural progenitor/stem cells (NSCs)<br>Improved locomotor function in parkinsonian rats<br>Increased TH/VMAT2-positive cells in the SN | [81] |
| Exendin-4 | 6-OHDA-induced PD model and LPS injected into the SNpc | 0.1 μg/kg and 0.5 μg/kg | Reduced amphetamine-induced rotations<br>Increased striatal dopamine and protected TH + neurons in SNpc in both models tested, reversing the loss of these neurons | [82] |
| Exendin-4 | MPTP-induced PD model | 10 μg/kg | Prevented loss of TH + SNpc neurons and TH + striatal fibers<br>Mechanism of neuroprotection is suggested by inhibition of microglial activation, reducing release of proinflammatory mediators | [83] |
| Exendin-4 | MPTP-induced PD model | 20 nM, 0.25 μl/h in the lateral ventricle | Neuroprotection over the dopaminergic system, because it prevented the loss of TH + SNpc neurons and TH + striatal fibers<br>Preserved dopaminergic levels and improved motor function in animals treated with MPTP | [84] |
| Exenatide | Single-blind trial design | 5-μg exenatide pen device (Byetta 5 μg) self-administrated twice-daily for 1 month, then 10 μg exenatide pen device (Byetta® 10 μg) self-administrated twice-daily for the subsequent 11 months | Improvement of motor and cognitive behavior as assessed by MDS-UPDRS scale after 12 months of treatment<br>Good tolerability | [87] |
| Exenatide | Single-center, randomized, double-blind, placebo-controlled trial | Subcutaneous injections of exenatide 2 mg once-weekly for 48 weeks | Treatment improved motor behavior as assessed by MDS-UPDRS scale | [88] |
| PT302 (long-acting Exendin-4 sustained release formulation) | 6-OHDA-induced PD model | 0.4 mg/kg and 2 mg/kg | Post-treatment of PT302 improved motor behavior and significantly increased TH + in the lesioned SN and striatum | [90] |

6-OHDA: 6-hydroxidopamine; LPS: lipopolysaccharides; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD: Parkinson's disease; SNpc: substantia nigra pars compacta. MDS-UPDRS: Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale.

## Concluding remarks

Medical and pharmaceutical alternatives for the treatment of neurodegenerative disorders are currently limited to the management of symptomatic manifestations. In the case of PD, the use of gold-standard medications causes a series of adverse effects through their continued and prolonged use. In light of this limitation, the search for new and effective compounds obtained from novel sources is imperative as a way to provide hope for patients and their families.

Scientific advances, especially in the development of omic analytical tools seeking the isolation, purification and identification of compounds, have evidenced the pharmaceutical potential found in the many bioactive components present in animal venoms. As discussed, these compounds possess antioxidant, anti-apoptotic, anti-inflammatory and neuroprotective properties, all of which are fundamental to target PD. Nevertheless, several of these promising and naturally occurring peptides possess restrictive intrinsic characteristics that hamper their human clinical development [91,92]. Strategies involving the use of rational peptide design, engineered nanomaterials (drug delivery systems), multifunctional and cell-penetrating peptides, peptide drug conjugates and technologies for alternative routes of administration are proposed to broaden the application of peptides as therapeutics by improving circulating plasma half-life, chemical and physical stability under physiological conditions, cell or tissue selectivity, molecular target specificity and oral bioavailability [91–93].

Counteracting limitations and generic pharmacokinetic disadvantages, >60 peptide drugs have been approved, >150 are in active clinical development and an additional 260 have been tested in human clinical trials [92]. However, the volume of data supporting laboratory positive results for PD does not translate into the current number of clinical trials testing animal venoms.

This failure to translate could be linked to an incomplete understanding of PD pathogenic mechanisms, excessive reliance on results obtained from toxin-based animal models, the absence of validated biomarkers of disease progression and/or unsuccessful trial design [94]. Thus, more research is needed to explore biochemical, genetic and pathological mechanisms involved in PD to offer a true differential therapeutic intervention for patients. We also foresee that, in the coming years, clinical development of these venom-derived peptides for PD will flourish, considering the emergence of peptide technologies, nanobiotechnological solutions and improved pharmaceutical properties that will further set the bases to ameliorate venom-derived therapeutic efficacy.

Overall, this review further supports the idea that the development of new adjunctive treatments for PD could be hidden in small and powerful venomous animals that use their poisonous weapons for a variety of purposes, many of which are still to be explored. We expect that the information presented in this paper and additional future discoveries in this field will awaken the interest of more researchers to invest in the discovery of new bioactive compounds isolated from animal venoms and explore their potential applications for the treatment of PD, bringing hope to patients who suffer from this ailment.

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