



Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en



Review article

Bee venom for the treatment of Parkinson's disease: How far is it possible?



Kamal Awad^{a,b,1}, Abdelrahman Ibrahim Abushouk^{a,c,d,1}, Ahmed Helal AbdelKarim^{a,b},
 Maged Mohammed^{a,b}, Ahmed Negida^{a,b,*}, Ali S. Shalash^e

^a Medical Research Group of Egypt, Cairo, Egypt

^b Faculty of Medicine, Zagazig University, Zagazig, Egypt

^c Faculty of Medicine, Ain Shams University, Cairo, Egypt

^d NovaMed Medical Research Association, Cairo, Egypt

^e Neurology Department, Ain Shams University, Cairo, Egypt

ARTICLE INFO

Article history:

Received 5 January 2017

Received in revised form 13 March 2017

Accepted 13 April 2017

Keywords:

Complementary therapies

Dopaminergic neurons

Bee venom

Parkinson's disease

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta leading to depletion of striatal dopamine and motor symptoms as bradykinesia, resting tremors, rigidity, and postural instability. Current therapeutic strategies for PD are mainly symptomatic and may cause motor complications, such as motor fluctuations and dyskinesia. Therefore, alternative medicine may offer an effective adjuvant treatment for PD. Bee venom therapy (BVT) has long been used as a traditional therapy for several conditions, such as rheumatoid arthritis, asthma, and skin diseases. Experimental and clinical studies showed that BVT could be an effective adjuvant treatment for PD. Several mechanisms were suggested for these findings including the ability of BVT to attenuate neuroinflammation, inhibit apoptosis of dopaminergic neurons, protect against glutamate-induced neurotoxicity, and restore normal dopamine levels in the nigrostriatal pathway. In this article, we reviewed and summarized the literature regarding the potential of BVT for the treatment of PD.

© 2017 Elsevier Masson SAS. All rights reserved.

Contents

1. Introduction	296
2. Mechanisms of action of bee venom in Parkinson's disease	296
2.1. Attenuation of neuroinflammation and microglial activation	296
2.2. Inhibition of apoptosis in dopaminergic neurons	297
2.3. Protection against glutamate-induced neurotoxicity	297
2.4. Restoration of normal brain neurochemistry	298
3. Evidence from experimental studies	299
4. Evidence from clinical studies	299
5. Future research on bee venom for Parkinson's disease	300
6. Authors' conclusions	300
Conflict of interest	300

Abbreviations: BV, bee venom; BVA, bee venom acupuncture; BVT, bee venom therapy; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; PD, Parkinson's disease; SN, substantia nigra; UPDRS, United Parkinson's Disease Rating Scale.

* Corresponding author at: Faculty of Medicine, Zagazig University, 44519, Zagazig, Egypt.

E-mail addresses: ahmed01251@medicine.zu.edu.eg, dr.negida@gmail.com

(A. Negida).

¹ These authors contributed equally to this work.

<http://dx.doi.org/10.1016/j.biopha.2017.04.065>

0753-3322/© 2017 Elsevier Masson SAS. All rights reserved.

Funding source	300
Acknowledgement	300
References	300

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD) [1–3], with a growing prevalence that reaches up to 3% in the population over 80 years [4]. Clinically, PD is characterized by cardinal motor symptoms, such as bradykinesia, resting tremors, rigidity, and postural instability [5], in addition to non-motor symptoms that include neuropsychiatric symptoms, sleep disorders, dysautonomia, gastrointestinal symptoms, and sensory complaints [6].

Pathologically, PD is characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and deposition of ubiquitinated misfolded proteins, known as Lewy bodies, in the cytoplasm of neurons [4,7–9]. The exact pathogenic mechanisms that cause the selective damage of dopaminergic neurons in PD patients are not completely understood. However, the current literature suggests that oxidative stress, neuroinflammation, mitochondrial dysfunction, and altered protein handling play a key role in the pathogenesis of PD [9–12].

Current treatment options for PD are mainly symptomatic and remain focused on motor symptoms only [13,14]. PD treatment targets normalizing the activity and chemical composition of the basal ganglia by restoring striatal dopamine levels [13]. Currently, L-dopa, a dopamine precursor, is one of the most effective drugs for PD [13,15]. However, after five years of levodopa treatment, about 50% of PD patients develop treatment-related complications, such as levodopa induced dyskinesia, motor fluctuations, hallucinations, and somnia [15–20]. Due to these limitations, investigators are searching for new therapeutic strategies for PD. There is an unmet need to find neuroprotective agents to slow the progress of PD and adjuvant drugs to decrease the need for levodopa and overcome its treatment-related complications [21].

Bee venom therapy (BVT) is a widespread alternative therapy that originated from the ancient China and Greece [22]. It contains many active substances (e.g. peptides, enzymes, and biogenic amines) with different pharmacological actions [22,23]. Although BV can cause neurotoxic and nociceptive effects, it also has anti-inflammatory [24,25], anti-nociceptive [26], radioprotective [27], and anti-mutagenic [28,29] effects. Therefore, it has been used as a traditional therapy for several diseases, such as rheumatoid arthritis, asthma, cancer, and skin diseases [30–32]. There are various methods to administer BVT: it can be applied as a cream, ointment, liniment, injection, acupuncture, or directly through a living bee [13,33]. However, bee venom acupuncture (BVA) is considered the most common mode of administration [13]. In terms of safety, no toxic effects of BV were noted when it was used at supratherapeutic levels (100 to 200 folds) [13,34].

Mellitin, a 26 aminoacid peptide, is the primary component of BV (40% to 60%) [35]. At high doses, it causes severe local pain and inflammation, while in lower doses, it can increase capillary permeability, exert anti-inflammatory effects, and lower blood pressure [36]. Since the early 1970s, studies have shown that mellitin can disrupt the brain's bioelectrical activity and inhibit the general behavior in animal models [22,37]. Moreover, it has been proven to inhibit apoptosis in SH-SY5Y cells and mitigate the inflammatory response of microglial cells [38].

Another component of BV is apamin (2% of BV content), an 18 aminoacid peptide, that is widely recognized as an irreversible blocker of Ca²⁺ activated K⁺ channels [35,39]. These channels are responsible for neuronal hyperpolarization and are found mainly

in AMPA and NMDA glutamatergic synapses [40]. Therefore, blocking these channels can reduce hyperpolarizing effects, enhance synaptic plasticity and memory functions [41,42]. Moreover, BV contains phospholipase A2 (PLA2), an enzyme that catalyzes the hydrolysis of membrane phospholipids, plays a vital role in signal transduction and regulates inflammatory responses [43].

Several studies showed the therapeutic potential of BV in multiple neurological diseases. In rat and mice models of multiple sclerosis, BV treatment was shown able to increase the count of T-regulator cells and suppress pro-inflammatory cytokine production; therefore, attenuating experimental autoimmune or allergic encephalomyelitis [44]. Moreover, in mutant superoxide dismutase-1 (hSOD1^{G93A}) transgenic mice, serving as models for amyotrophic lateral sclerosis (ALS), subcutaneous BV acupuncture (at a dose of 0.1 µg/g—three times/week for two weeks) improved the mice motor activity on the rotarod test and increased the lifespan of treated mice [45,46]. In AD mice models, treatment by BV-PLA2 improved cognitive functions and cerebral glucose metabolism and reduced the levels of hippocampal amyloid-Beta deposits [47].

Recently, multiple experimental studies have investigated the anti-neuroinflammatory effects of BVT as a possible treatment for PD, and interestingly, these studies showed promising results. In this article, we aimed at summarizing and reviewing the literature regarding the potential use of BVT for the treatment of PD.

2. Mechanisms of action of bee venom in Parkinson's disease

2.1. Attenuation of neuroinflammation and microglial activation

Abnormal microglial activation is a pathological hallmark in neurodegenerative diseases, including PD and AD [48]. Experimental activation of BV2 microglial cells (brain microglial cells from C57BL/6 mice), using bacterial lipopolysaccharide (LPS), increases the expression of tumor necrosis factor (TNF-α) and inducible nitric oxide synthase (iNOS), promoting neuroinflammation and nitric oxide (NO)-mediated neuronal death [49]. Moreover, activated microglia can generate superoxide free radicals via activation of NADPH oxidase enzyme [50,51]. It is also believed that activated microglia play a role in dopaminergic terminal loss in early PD [52].

Several preclinical trials examined whether BV can attenuate leukocyte migration or microglial activation in cellular and animal models. Moon et al. reported the ability of BV and its peptide (mellitin) to inhibit the expression of NO and iNOS in LPS-stimulated BV2 microglia in a dose-dependent manner [53]. Yang et al. showed that pretreatment of mutant hSOD1 transgenic mice (serving as an animal model for ALS) suppressed the expression of microglial markers in the brain stem and spinal cord [45]. Moreover, Jang et al. suggested that BV can prevent microglial activation and leukocyte migration through its antibacterial effect, removing a possible source of microglial stimulation [54]. In another animal study by Chung et al., BV reduced brain tissue infiltration with CD4T cells through increasing the proportion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Tregs), which play an essential role in maintaining immune tolerance under physiological conditions [55].

The recent literature suggests that oxidative stress plays a central role in the pathogenesis of PD [56]. Neuroinflammation

generates reactive oxygen species (ROS) that cause oxidative damage, especially in dopaminergic neurons due to their high content of dopamine and lipids [57,58]. Another study showed that paraoxonase (PON-1), an HDL-associated enzyme that protects membranes against lipid peroxidation [59], is deficient in PD patients and rotenone-induced PD models [60]. In a preclinical trial by Khalil et al., BVA protected against oxidative damage in MPTP and rotenone-induced PD mouse models through reduction of lipid peroxides brain levels and normalizing the antioxidant activities of glutathione (GSH) and PON-1 enzyme [61]. Another anti-inflammatory mechanism of BV is inhibiting cyclooxygenase-II enzyme activity, reducing the production of pro-inflammatory cytokines [54]. Moreover, mellitin was shown able to reduce tissue degradation [62] through inhibition of Phospholipase A1 enzyme [63]. These effects are further augmented by BV-induced transcriptional downregulation of nuclear factor kappa B (NF- κ B) [53], which is involved in a pro-inflammatory signaling pathway that promotes the expression of inflammatory cytokines [64].

2.2. Inhibition of apoptosis in dopaminergic neurons

Several studies suggested that apoptosis plays a role in neuronal cell death in neurodegenerative diseases, including PD [65,66]. Increased levels of key apoptotic regulator and effector factors, such as Bax and caspase-3 have been consistently reported in PD models [67]. Preclinical studies revealed that BV components are able to maintain the survival of dopaminergic neurons and improve locomotor behavior in PD animal models through enhancing the prosurvival pathways or inhibition of the proapoptotic pathways. Khalil et al. showed that treatment with BVA suppressed the expression of caspase-3 and Bax apoptotic genes in rotenone-induced PD models [61]. Poly ADP-ribose polymerase-1 (PARP-1) is an abundant nuclear enzyme that is physiologically involved in DNA repair. However, overactivation of PARP-1 upon

cleavage by caspase-3 can promote cell death [68]. Doo et al. showed that BV treatment inhibited PARP cleavage in MPP⁺-intoxicated SH-SY5Y cells [69].

On the other hand, the activation of phosphatidylinositol 3-kinase (PI3K) promotes cell survival through its downstream target (Akt), which inhibits gene expression of Bax/Bcl-2 apoptosis mediators [70]. Two studies by Doo et al. reported that BV treatment in MPP⁺-intoxicated SH-SY5Y cells and MPTP-induced mouse PD model enhanced the PI3K/Akt prosurvival pathway, rescuing dopaminergic cells from apoptosis [69,71].

2.3. Protection against glutamate-induced neurotoxicity

Being an excitatory neurotransmitter, glutamate is involved in physiologic basal ganglia circuitry (17). However, with dopamine depletion, glutamatergic signals from the subthalamic nucleus become hyperactive, impairing the extrapyramidal motor control (18). Moreover, glutamate can act as a neurotoxin if the mitochondrial energy metabolism is impaired (19). Alterations in glutamate release and uptake, as well as overstimulation of NMDA receptors, is a part of the pathogenesis of several neurodegenerative diseases, such as PD, AD and ALS [72]. It may also explain the neurodegenerative changes, observed following hypoglycemia, hypoxia, or seizures [73]. Several signaling pathways are involved in glutamate-mediated neurotoxicity, such as extracellular signal-regulated kinase (ERK), alpha serine/threonine-protein kinase 1 (AKT), p38, and c-Jun N-terminal kinase (JNK) [74,75].

Lee et al. conducted an in-vitro study to investigate if pretreatment with BV can protect against glutamate-mediated neurotoxicity in N2a neuroblastoma and BV2 microglial cells. They observed a dose-dependent glutamate-mediated neurotoxicity in cultured cells, which was primarily induced by activation of protein kinase C (PKC) enzyme, leading to phosphorylation and activation of ERK, p38, and JNK. The authors also reported that

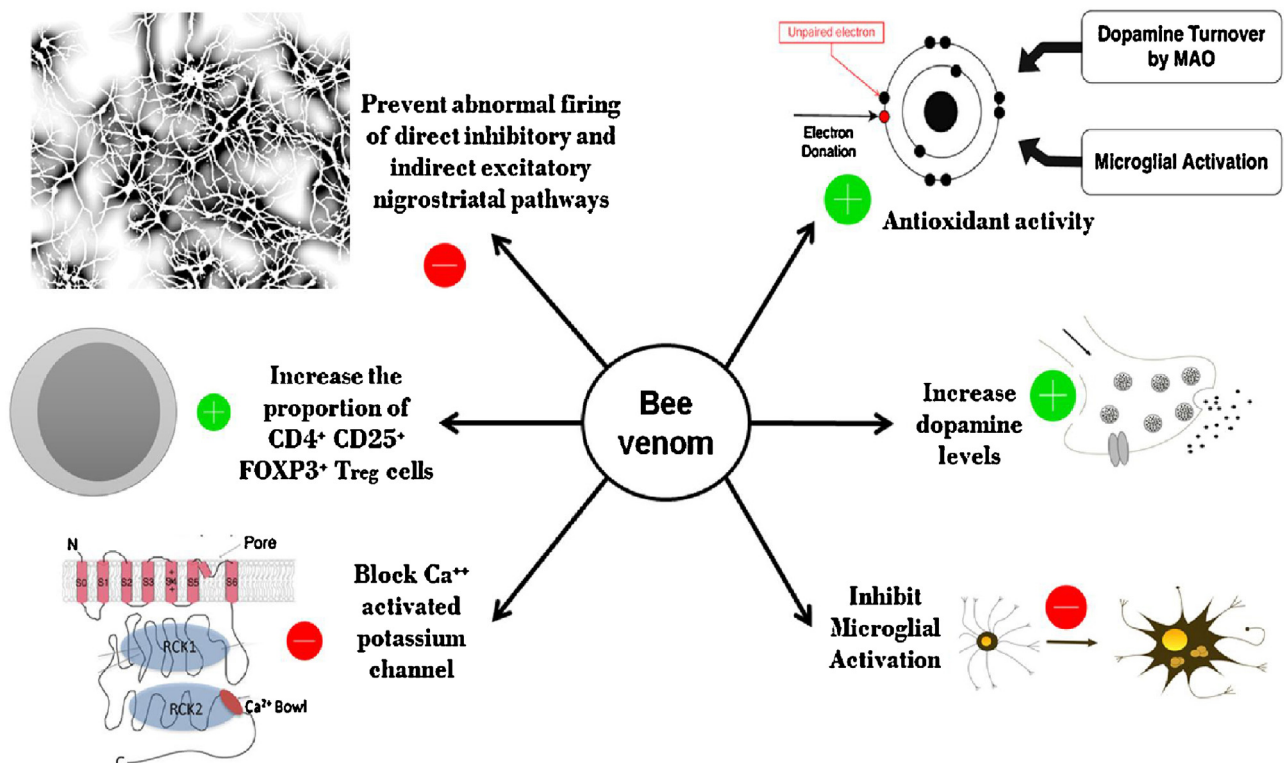


Fig. 1. Shows possible mechanisms of action of bee venom as a therapeutic agent for Parkinson's disease.

pretreatment of these cells with BV inhibited phosphorylation of these proteins and subsequent cell death. Moreover, BV at a concentration of 2.5 $\mu\text{g/mL}$ increased phospho-AKT expression, a signaling pathway that enhances cellular survival [76].

2.4. Restoration of normal brain neurochemistry

The major pathological hallmark of PD is deficiency of dopamine in the SN and associated neuronal circuitry. Khalil et al. showed that BVA reversed the depletion of dopamine, serotonin, and noradrenaline after rotenone intoxication in mice

[61]. These findings cannot be attributed to acupuncture itself because other studies reported that pure acupuncture could not increase the brain dopamine levels [77,78]. Thomas et al. showed that BV can also increase noradrenaline levels, which is associated with improved motor functions. This elevation of noradrenaline brain levels may be a secondary effect of increasing brain dopamine levels, which is the main precursor for noradrenaline synthesis [79].

There is evidence that BV components can affect the transduction of electrical signals between neurons through modulating the activity of ion pumps and channels. Alvarez-Fischer et al. reported

Table 1
summarizes the design of preclinical

Author, Year	Animal model	Dosage	Experimental design	Outcomes and possible mechanisms
Doo et al. 2010 [71]	MPTP induced-mouse model of PD (Male C57BL/6 mice)	Bee venom (BV) (0.02 ml)	Mice were divided into three groups: (1) control group receiving saline, (2) MPTP-intoxicated group, and (3) MPTP-intoxicated group, pretreated with bee venom acupuncture (BVA) for two weeks. Tyrosine hydroxylase (TH) immunohistochemistry was used to evaluate loss of dopaminergic neurons in the substantia nigra (SN).	Treatment with BVA protected dopaminergic neurons from MPTP neurotoxicity and attenuated MPTP induced phospho-Jun immunoreactivity in the SN.
Kim et al. 2011 [83]	MPTP induced-mouse model of PD (Male C57BL/6 mice)	0.6 mg/kg of BV in 20 μL of normal saline	Mice were divided into four groups: (1) MPTP-intoxicated mice, (2) MPTP-intoxicated mice, treated with subcutaneous BV injection, (3) MPTP-intoxicated mice, treated with saline, and (4) Normal mice treated with BV injection. Dopaminergic neuronal loss in the SN was evaluated by TH immunohistochemistry and microglial activation was measured by inducible nitric oxide synthase (iNOS) and macrophage antigen complex-1 (MAC-1) immunohistochemistry.	Treatment with BV injection improved survival rates of dopaminergic neurons in the SN and attenuated microglial activation and expression of inflammatory markers, such as iNOS and MAC-1 and proinflammatory enzymes, such as cyclooxygenase-2 and phospholipase A1.
Chung et al. 2012 [55]	MPTP induced-mouse model of PD (Male C57BL/6 mice)	BV (1 mg/kg)	Mice were divided into four groups. Two groups received BV or vehicle alone. The other two groups received four intraperitoneal (i.p) injections of MPTP at two hours interval. Twelve hours later, mice received i.p injections of BV or phosphate-buffered saline (PBS) injection, once daily for six days.	Treatment with BV treatment prevented MPTP-induced neuronal loss in the SN. It also inhibited microglial activation and CD-4 T cell infiltration, which are underlying mechanisms for MPTP neurotoxicity. This occurred through increasing the proportion of CD4 ⁺ CD25 ⁺ Foxp3 ⁺ regulatory T cells (Tregs), which play a vital role in maintaining normal immune tolerance.
Alvarez-Fischer et al. 2013 [80]	MPTP/Probenecid induced-mouse model of PD (Male C57BL/6 mice)	BV (12–120 mg/kg) or apamin (0.5–1 mg/kg)	Mice were divided into four groups: (1) Control group receiving vehicle only, (2) MPTP/Probenecid group receiving 10 i.p injections over five weeks, (3) MPTP-intoxicated mice receiving BV at low/high doses, and (4) MPTP-intoxicated mice receiving apamin at low/high doses.	Treatment with BV prevented MPTP-induced loss of dopaminergic neurons and increased striatal dopamine levels in a dose-dependent manner. Apamin reproduced these effects partially, indicating the presence of other neuroprotective compounds in BV.
Maurice et al. 2015 [81]	Pharmacological (i.p. haloperidol) and lesional (unilateral intranigral 6-hydroxydopamine injection) PD models (Male Wistar rats).	BV (1 or 3 mg/kg) or apamin (0.1, 0.2, or 0.4 mg/kg)	Rats received i.p. haloperidol injections, followed by injections of BV, apamin, CyPPA (cyclohexyl-[2-(3, 5-dimethylpyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine), or BV with CyPPA. Then, cortical stimulation-evoked responses were evaluated by electrophysiology. Another group of mice received unilateral intranigral 6-hydroxydopamine injection. After 15 to 21 days post-surgery, animals received i.p. injections of BV or saline. Then, both groups underwent behavioral analysis using the cylinder test and modulation of apomorphine-induced behavioral changes.	In hemi-parkinsonian, 6-hydroxydopamine-induced, rat model, BV or apamin treatment reduced contralateral forelimb akinesia. Moreover, it reversed haloperidol-induced catalepsy, a model of parkinsonian akinetic deficit. The antiparkinsonian effect of BV is partially mediated by blocking (small conductance Ca ²⁺ activated) K ⁺ channels and preventing abnormal firing in the direct inhibitory and indirect excitatory nigrostriatal pathways. These effects were blocked by CyPPA, an activator of small conductance Ca ²⁺ activated K ⁺ channels.
Khalil et al. 2015 [61]	Rotenone induced PD model (Male Swiss mice)	BV (0.02 ml once every three days for two weeks)	Mice were divided into four groups that received: (1) normal saline, (2) rotenone, (3) rotenone plus L-dopa (25 mg/kg, daily, orally for six days), or (4) rotenone plus BVA.	Treatment with BVA reversed the rotenone-induced depletion of dopamine, serotonin, and noradrenaline. It also improved the antioxidant pool of the brain tissue and suppressed the expression of proapoptotic caspase-3 and Bcl-2 genes.

Abbreviations: BV: Bee venom, BVA: Bee venom acupuncture, CyPPA: (cyclohexyl-[2-(3, 5-dimethylpyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine), MPTP: 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, PD: Parkinson's disease, SN: Substantia nigra, TH: Tyrosine hydroxylase.

that apamin can block small-conductance (Ca^{+2} activated) K^{+} channels, reserving dopaminergic neurons in MPTP/probenecid-intoxicated mice [80,81]. In another in-vitro study by Salthun-Lassalle et al., melittin was shown able to inhibit the activity of Na/K-ATPase pump, increasing neuronal excitability and adding protection to dopaminergic neurons by a mechanism involving T-type calcium channel activation [82]. Possible mechanisms of action of BV in PD are illustrated in Fig. 1.

3. Evidence from experimental studies

Many experimental studies [55,61,71,80,81,83] investigated the efficacy of BVT for PD. Khalil et al. [61] performed an experimental study on rotenone-induced PD model (Male Swiss mice) using BV in a dose of 0.02 ml once every three days for two weeks. Treatment with BVA reversed the rotenone-induced depletion of dopamine, norepinephrine, and serotonin. It also enhanced the antioxidant pool of the brain tissue and suppressed the expression of proapoptotic caspase-3 and Bcl-2 genes. Similar positive findings were achieved on other animal models of PD, such as MPTP mice [55,71,80,83] and 6-hydroxydopamine-induced rat model [81]. A summary of the dosage, experimental design, and key findings of these studies is shown in Table 1.

4. Evidence from clinical studies

In 2012, Cho et al. [84] conducted a pilot randomized controlled trial (RCT) in which 43 adults with idiopathic PD were divided into three groups: (I) acupuncture, (II) BVA, and (III) control groups. All subjects have been on a stable anti-parkinsonian drug for at least

one month, and they received the treatments two times weekly for eight weeks. The results showed significant improvements in the BVA group on the total Unified Parkinson's Disease Rating Scale (UPDRS), UPDRS part II, UPDRS part III, the Berg Balance Scale (BBS), and the 30 m walking time in comparison to the control group. Also, significant improvements were shown in the acupuncture group on the total UPDRS, UPDRS III, and Beck Depression Inventory (BDI). No significant changes were seen in the control group.

In another trial [85], 11 patients with idiopathic PD received a conventional anti-parkinsonian treatment for 12 weeks then, received acupuncture combined with BV as an adjuvant therapy to the conventional treatment twice a week for another 12 weeks. In comparison to the conventional treatment, significant improvements were seen after adding adjuvant therapies on UPDRS II, UPDRS III, gait speed, and Parkinson's disease Quality of Life Questionnaire (PDQL). In addition, the combination therapy was safe and well-tolerated.

In a recent RCT, Hartmann et al. [86] investigated the potential therapeutic and neuroprotective effects of BV injections, in comparison to placebo, in patients with PD at Hoehn & Yahr stages 1.5 to 3. Forty patients were randomized to receive either (1) BV injection [100 μg] or (2) an equivalent volume of saline (placebo) once monthly for 11 months. No significant effects of BV were seen on the UPDRS III scores in the "off" condition or on nuclear imaging. Compared to the previous trials, the lack of clinical benefit in this study might be explained by: (1) The small individual doses and low administration frequency of BVT, (2) In the above two studies, BV was applied at acupuncture points; therefore, the clinical effect may be due to the combination of

Table 2

shows a summary of human trials that investigated the safety and efficacy of bee venom therapy in PD patients.

Study	Country	Design	Interventions	Frequency of treatment and Duration	Patients	Outcome measure	Baseline	Endpoint
Cho et al. 2012 [84]	Korea	Randomized controlled trial	Acupuncture versus BVA versus control	Twice a week for eight weeks.	Forty-three adults with idiopathic PD who had been on a stable dose of antiparkinsonian medication for at least one month	Total UPDRS UPDRS II UPDRS III BBS 30-m walking time (sec)	32.0 (21.5, 51.0) 9.0 (6.0, 17.0) 15.0 (10.0, 18.5) 55.0 (52.0, 56.0) 27.69 (26.53, 29.98)	24.0 (17.5, 35.0) [‡] 7.0 (5.0, 11.5) [‡] 10.0 (6.5, 10.0) [‡] 56.0 (55.5, 56.0) [‡] 26.13 (24.65, 28.47) [‡]
Doo et al. 2015 [85]	Korea	Prospective open-label clinical study	Conventional treatment versus conventional treatment + acupuncture and BVA as an adjunctive treatment	Conventional treatment for 12 weeks. Subsequently, they received additional treatment with acupuncture and BVA twice weekly for twelve weeks	Eleven patients (seven men and four women) with idiopathic PD who had been receiving a stable dose of anti-parkinsonian medication for at least four weeks	UPDRS II UPDRS III PDQL score Gait speed	14 (11, 17) 17 (13, 20) 122 (105, 132) 21 (19, 25)	12 (10, 14) [‡] 15 (12, 16) [‡] 147 (144, 160) [‡] 19 (17, 23) [‡]
Hartmann et al. 2016 [86]	France	Randomized controlled trial	BV injections versus equivalent volumes of saline	once a month over an 11 month periods	Forty PD patients at Hoehn & Yahr stages 1.5 to 3	UPDRS II UPDRS III	−0.03 (0.08) ^{‡,†} 0.013 (0.04) ^{‡†}	

Abbreviations: BVA = bee venom acupuncture; PD = Parkinson's disease UPDRS = Unified Parkinson's Disease Rating Scale; BBS = Berg Balance Scale; PDQL = Parkinson's Disease Quality of Life Questionnaire. All values are median (lower quartile, upper quartile).

[‡] Indicates a statistical difference between treatment group and the control group (p value < 0.05).

[†] Data are presented with estimates of monthly variation and (its standard error).

[‡] Indicates a non-significant difference between treatment group and the control group (p value > 0.05).

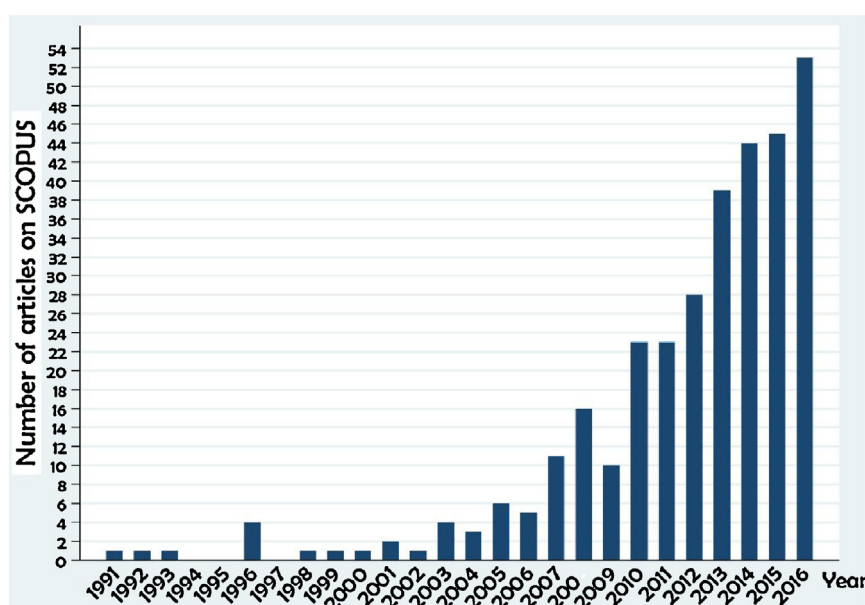


Fig. 2. Shows the frequency of SCOPUS indexed publications on bee venom therapeutic use in Parkinson's disease.

acupuncture with BV, and (3) Difference in the severity of PD between BV and control groups: patients in the BV group were more affected than those in the control group. A summary of the design and key findings of these trials is shown in Table 2.

5. Future research on bee venom for Parkinson's disease

This review shows that BV should be further investigated as a possible adjuvant treatment for PD. Despite the positive findings of the experimental and early clinical studies, there are only few published data regarding the efficacy of BV for PD. Our systematic search on PubMed retrieved 35 records. Another search on SCOPUS using the term (Bee venom AND Parkinson's disease) yielded 323 documents. The frequency of publications in this area since 1991 to date is shown in Fig. 2.

Interestingly, the figure shows an increasing publication frequency in this area; the number of recorded publications has doubled since 2010 ($n=23$) till 2016 ($n=53$). However, based on our review of the literature, current evidence is not sufficient to confirm the efficacy of this treatment strategy. Moreover, we could not employ the methods of evidence synthesis in health research (systematic review and meta-analysis) because the number of available clinical trials was limited, giving insufficient basis to conduct such analysis.

As we mentioned earlier, there is an unmet clinical need to develop adjuvant treatments to improve patients' response to the antiparkinsonian medications and to reduce the levodopa-related complications. Therefore, we believe that BV is a good candidate for future clinical evaluations.

Further well designed, large RCTs are recommended to: (1) define the minimal required and the most effective doses and administration frequencies of BVT; (2) investigate its long-term effects on the progression of PD because most published studies reported short term outcomes only; (3) compare its effects when combined with conventional treatments against the conventional treatments alone in different stages of PD; and (4) define the most effective mode of administration. The promising effects of complementary medicine therapeutics should be further advocated and communicated to practicing neurologists and other healthcare professionals [87,88].

6. Authors' conclusions

Based on the available experimental and clinical studies on BVT for PD, we conclude that BVT has a favorable safety profile and might be a promising adjuvant treatment for PD. Future RCTs are warranted with a larger sample size and higher dose and frequency of administration.

IDENTIFICATION OF STUDIES FOR THIS REVIEW

We searched PubMed from inception till November, 2016 using the following combination of keywords and MESH terms ("bee venoms"[MeSH Terms] OR ("bee"[All Fields] AND "venoms"[All Fields]) OR "bee venoms"[All Fields] OR ("bee"[All Fields] AND "venom"[All Fields]) OR "bee venom"[All Fields]) AND ("parkinson disease"[MeSH Terms] OR ("parkinson"[All Fields] AND "disease"[All Fields]) OR "parkinson disease"[All Fields] OR ("Parkinson's"[All Fields] AND "disease"[All Fields]) OR "Parkinson's disease"[All Fields]). Studies were screened for relevance to the concept of our review. Relevant studies were divided into preclinical and clinical studies as described in Tables 1 and 2; respectively.

Conflict of interest

None to declare.

Funding source

None to declare.

Acknowledgement

We would like to thank the members of Medical Research Group of Egypt (MRGE: www.mrg-egypt.org) for their support and encouragement. Ahmed Negida would like to thank Kamal Awad for the commitment and dedication in preparing this article.

References

- [1] R. Mayeux, Epidemiology of neurodegeneration, *Annu. Rev. Neurosci.* 26 (2003) 81–104, doi:<http://dx.doi.org/10.1146/annurev.neuro.26.043002.094919>.
- [2] L.M.L. De Lau, M.M.B. Breteler, Epidemiology of parkinson's disease, *Lancet Neurol.* 5 (2006) 525–535.

- [3] S. Fahn, D. Sulzer, Neurodegeneration and neuroprotection in Parkinson disease, *NeuroRx: J. Am. Soc. Exp. Neurother.* 1 (2004) 139–154, doi:http://dx.doi.org/10.1602/neuroRx.1.1.139.
- [4] A.E. Guttmacher, F.S. Collins, R.L. Nussbaum, C.E. Ellis, Alzheimer's disease and parkinson's disease, *New Engl. J. Med.* 348 (2003) 1356–1364, doi:http://dx.doi.org/10.1056/NEJM2003ra020003.
- [5] M. Hoehn, M. Yahr, Parkinsonism: onset, progression, and mortality, *Neurology* 50 (1998) 318.
- [6] K.R. Chaudhuri, D.G. Healy, A.H. Schapira, National Institute for Clinical Excellence, Non-motor symptoms of Parkinson's disease: diagnosis and management, *Lancet Neurol.* 5 (2006) 235–245, doi:http://dx.doi.org/10.1016/S1474-4422(06)70373-8.
- [7] S. Kuzuhara, H. Mori, N. Izumiya, et al., Lewy bodies are ubiquitinated, *Acta Neuropathol. (Berl.)* 75 (1988) 345–353, doi:http://dx.doi.org/10.1007/BF00687787.
- [8] M.S. Pollanen, D.W. Dickson, C. Bergeron, Pathology and biology of the Lewy body, *J. Neuropathol. Exp. Neurol.* 52 (1993) 183–191.
- [9] R.L. Mosley, E.J. Benner, I. Kadriu, et al., Neuroinflammation, oxidative stress and the pathogenesis of parkinson's disease, *Clin. Neurosci. Res.* 6 (2006) 261–281, doi:http://dx.doi.org/10.1016/j.cnr.2006.09.006.
- [10] J.T. Greenamyre, T.G. Hastings, BIOMEDICINE: Parkinson's-divergent causes, convergent mechanisms, *Science* 304 (2004) 1120–1122, doi:http://dx.doi.org/10.1126/science.1098966.
- [11] A.H. Schapira, P. Jenner, Etiology and pathogenesis of Parkinson's disease, *Mov. Disord.* 26 (2011) 1049–1055, doi:http://dx.doi.org/10.1002/mds.23732.
- [12] O. Hwang, Role of oxidative stress in parkinson's disease, *Exp. Neurobiol.* 22 (2013) 11, doi:http://dx.doi.org/10.5607/en.2013.22.1.11.
- [13] J. Silva, V. Monge-Fuentes, F. Gomes, et al., Pharmacological alternatives for the treatment of neurodegenerative disorders: wasp and bee venoms and their components as new neuroactive tools, *Toxins* 7 (2015) 3179–3209, doi:http://dx.doi.org/10.3390/toxins7083179.
- [14] S. Li, J. Dong, C. Cheng, W. Le, Therapies for Parkinson's diseases: alternatives to current pharmacological interventions, *J. Neural Transm.* 123 (2016) 1279–1299, doi:http://dx.doi.org/10.1007/s00702-016-1603-9.
- [15] L. Verhagen Metman, Recognition and treatment of response fluctuations in Parkinson's disease: review article, *Amino Acids* 23 (2002) 141–145, doi:http://dx.doi.org/10.1007/s00726-001-0119-1.
- [16] T.P.S. Group, Levodopa and the progression of parkinson's disease, *New Engl. J. Med.* 351 (2004) 2498–2508, doi:http://dx.doi.org/10.1056/NEJMoa033447.
- [17] O. Rascol, D.J. Brooks, A.D. Korczyn, et al., A five-year study of the incidence of dyskinesia in patients with early parkinson's disease who were treated with ropinirole or levodopa, *New Engl. J. Med.* 342 (2000) 1484–1491, doi:http://dx.doi.org/10.1056/NEJM200005183422004.
- [18] R. Kurlan, Levodopa phobia: a new iatrogenic cause of disability in Parkinson disease, *Neurology* 64 (923) (2005) 2–924, doi:http://dx.doi.org/10.1212/01.WNL.0000152880.77812.5B.
- [19] S. Sharma, S. Singh, V. Sharma, et al., Neurobiology of L-DOPA induced dyskinesia and the novel therapeutic strategies, *Biomed. Pharmacother.* 70 (2015) 283–293, doi:http://dx.doi.org/10.1016/j.biopha.2015.01.029.
- [20] A.K. Kakkar, N. Dahiya, Management of Parkinson's disease: current and future pharmacotherapy, *Eur. J. Pharmacol.* 750 (2015) 74–81, doi:http://dx.doi.org/10.1016/j.ejphar.2015.01.030.
- [21] J.M. Savitt, V.L. Dawson, T.M. Dawson, Diagnosis and treatment of Parkinson disease: molecules to medicine, *J. Clin. Invest.* 116 (2006) 1744–1754.
- [22] D.J. Son, J.W. Lee, Y.H. Lee, et al., Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds, *Pharmacol. Ther.* 115 (2007) 246–270, doi:http://dx.doi.org/10.1016/j.pharmthera.2007.04.004.
- [23] D.-S. Hwang, S. Kim, H. Bae, Therapeutic effects of bee venom on immunological and neurological diseases, *Toxins* 7 (2015) 2413–2421, doi:http://dx.doi.org/10.3390/toxins7072413.
- [24] J.Y. Lee, S.S. Kang, J.-H. Kim, et al., Inhibitory effect of whole bee venom in adjuvant-induced arthritis, *In vivo (Athens, Greece)* 19 (2005) 801–805.
- [25] Y.C. Park, P.S. Koh, B.K. Seo, et al., Long-Term effectiveness of bee venom acupuncture and physiotherapy in the treatment of adhesive capsulitis: a one-Year follow-Up analysis of a previous randomized controlled trial, *J. Altern. Complem. Med.* 20 (2014) 919–924, doi:http://dx.doi.org/10.1089/acm.2014.0220.
- [26] H. Yoon, M.J. Kim, I. Yoon, et al., Nicotinic acetylcholine receptors mediate the suppressive effect of an injection of diluted bee venom into the GV3 acupoint on oxaliplatin-Induced neuropathic cold allodynia in rats, *Biol. Pharm. Bull.* 38 (2015) 710–714, doi:http://dx.doi.org/10.1248/bpb.b14-00797.
- [27] G. Gajski, V. Garaj-Vrhovac, Radioprotective effects of honeybee venom (*Apis mellifera*) against 915-MHz microwave radiation-Induced DNA damage in wistar rat lymphocytes: In vitro study, *Int. J. Toxicol.* 28 (2009) 88–98, doi:http://dx.doi.org/10.1177/1091581809335051.
- [28] J.-E. Huh, Y.-H. Baek, M.-H. Lee, et al., Bee venom inhibits tumor angiogenesis and metastasis by inhibiting tyrosine phosphorylation of VEGFR-2 in LLC-tumor-bearing mice, *Cancer Lett.* 292 (2010) 98–110, doi:http://dx.doi.org/10.1016/j.canlet.2009.11.013.
- [29] H. Hu, D. Chen, Y. Li, X. Zhang, Effect of polypeptides in bee venom on growth inhibition and apoptosis induction of the human hepatoma cell line SMMC-7721 in-vitro and Balb/c nude mice in-vivo, *J. Pharm. Pharmacol.* 58 (2006) 83–89, doi:http://dx.doi.org/10.1211/jpp.58.1.0010.
- [30] M.S. Choi, S. Park, T. Choi, et al., Bee venom ameliorates ovalbumin induced allergic asthma via modulating CD4 + CD25+ regulatory T cells in mice, *Cytokine* 61 (2013) 256–265, doi:http://dx.doi.org/10.1016/j.cyt.2012.10.005.
- [31] J.-D. Lee, H.-J. Park, Y. Chae, S. Lim, An overview of bee venom acupuncture in the treatment of arthritis, *Evid Based Complement Alternat Med.: eCAM* 2 (2005) 79–84, doi:http://dx.doi.org/10.1093/ecam/neh070.
- [32] R.C. Hider, Honeybee venom: a rich source of pharmacologically active peptides, *Endeavour* 12 (1988) 60–65.
- [33] M.A.A.-S.M. Ali, M.A.A.-S. Mohamed, Studies on bee venom and its medical uses, *Int. J. Advanc. Res. Technol.* 1 (2) (2012) 69–83.
- [34] H.-W. Kim, Y.-B. Kwon, T.-W. Ham, et al., General pharmacological profiles of bee venom and its water soluble fractions in rodent models, *J. Vet. Sci.* 5 (2004) 309–318.
- [35] M. Moreno, E. Giralt, Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan, *Toxins* 7 (2015) 1126–1150.
- [36] H. Raghuraman, A. Chattopadhyay, Melittin: a membrane-active peptide with diverse functions, *Biosci. Rep.* 27 (2007) 189–223.
- [37] N.K. Vyatchannikov, A.Y. Sinka, The effect of melittin-the major constituent of the bee venom-on the central nervous system, *Farmakologiya i Toksikologiya* 36 (1973) 526–530.
- [38] E.J. Yang, S.H. Kim, S.C. Yang, et al., Melittin restores proteasome function in an animal model of ALS, *J. Neuroinflammation* 8 (2011) 69.
- [39] C. Lamy, S.J. Goodchild, K.L. Weatherall, et al., Allosteric block of KCa2 channels by apamin, *J. Biol. Chem.* 285 (2010) 27067–27077.
- [40] J. Lam, N. Coleman, A.L.A. Garing, H. Wulff, The therapeutic potential of small-conductance KCa2 channels in neurodegenerative and psychiatric diseases, *Expert Opin. Ther. Targets* 17 (2013) 1203–1220.
- [41] R.W. Stackman, R.S. Hammond, E. Linardatos, et al., Small conductance Ca2 +-activated K+ channels modulate synaptic plasticity and memory encoding, *J. Neurosci.* 22 (2002) 10163–10171.
- [42] C.D.C. Gati, M.R. Mortari, E.F. Schwartz, Towards therapeutic applications of arthropod venom K_v J. Toxicol. 2012 (2012).
- [43] J.E. Burke, E.A. Dennis, Phospholipase A2 structure/function, mechanism, and signaling, *J. Lipid Res.* 50 (2009) S237–S242.
- [44] A. Karimi, F. Ahmadi, K. Parivar, et al., Effect of honey bee venom on lewis rats with experimental allergic encephalomyelitis, a model for multiple sclerosis, *Iran. J. Pharm. Res.* 11 (2012) 671–678.
- [45] E.J. Yang, J.H. Jiang, S.M. Lee, et al., Bee venom attenuates neuroinflammatory events and extends survival in amyotrophic lateral sclerosis models, *J. Neuroinflammation* 7 (2010) 1.
- [46] M. Cai, S.-M. Choi, E.J. Yang, The effects of bee venom acupuncture on the central nervous system and muscle in an animal hSOD1G93A mutant, *Toxins* 7 (2015) 846–858.
- [47] M. Ye, H.-S. Chung, C. Lee, et al., Neuroprotective effects of bee venom phospholipase A2 in the 3xTg AD mouse model of Alzheimer's disease, *J. Neuroinflammation* 13 (2016) 10.
- [48] F. Aloisi, The role of microglia and astrocytes in CNS immune surveillance and immunopathology, *The Functional Roles of Glial Cells in Health and Disease*, Springer, 1999, pp. 123–133.
- [49] T. Arimoto, G. Bing, Up-regulation of inducible nitric oxide synthase in the substantia nigra by lipopolysaccharide causes microglial activation and neurodegeneration, *Neurobiol. Dis.* 12 (2003) 35–45.
- [50] H.-M. Gao, B. Liu, W. Zhang, J.-S. Hong, Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease, *FASEB J.* 17 (2003) 1954–1956.
- [51] D.-C. Wu, P. Teismann, K. Tieu, et al., NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine model of Parkinson's disease, *Proc. Natl. Acad. Sci.* 100 (2003) 6145–6150.
- [52] Y. Ouchi, E. Yoshikawa, Y. Sekine, et al., Microglial activation and dopamine terminal loss in early Parkinson's disease, *Ann. Neurol.* 57 (2005) 168–175.
- [53] D.-O. Moon, S.-Y. Park, K.-J. Lee, et al., Bee venom and melittin reduce proinflammatory mediators in lipopolysaccharide-stimulated BV2 microglia, *Int. Immunopharmacol.* 7 (2007) 1092–1101.
- [54] H.-S. Jang, S.K. Kim, J.-B. Han, et al., Effects of bee venom on the pro-inflammatory responses in RAW264. 7 macrophage cell line, *J. Ethnopharmacol.* 99 (2005) 157–160.
- [55] E.S. Chung, H. Kim, G. Lee, et al., Neuro-protective effects of bee venom by suppression of neuroinflammatory responses in a mouse model of Parkinson's disease: role of regulatory T cells, *Brain Behav. Immun.* 26 (2012) 1322–1330.
- [56] J. Sian, D.T. Dexter, A.J. Lees, et al., Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia, *Ann. Neurol.* 36 (1994) 348–355.
- [57] R.L. Miller, M. James-Kracke, G.Y. Sun, A.Y. Sun, Oxidative and inflammatory pathways in Parkinson's disease, *Neurochem. Res.* 34 (2009) 55–65.
- [58] E. Milusheva, M. Baranyi, E. Kormos, et al., The effect of antiparkinsonian drugs on oxidative stress induced pathological [3 H] dopamine efflux after in vitro rotenone exposure in rat striatal slices, *Neuropharmacology* 58 (2010) 816–825.
- [59] M. Rosenblat, M. Aviram, Paraoxonases role in the prevention of cardiovascular diseases, *Biofactors* 35 (2009) 98–104.
- [60] K. Ikeda, Y. Nakamura, T. Kiyozuka, et al., Serological profiles of urate, paraoxonase-1, ferritin and lipid in Parkinson's disease: changes linked to disease progression, *Neurodegenerative Dis.* 8 (2011) 252–258.

- [61] W.K.B. Khalil, N. Assaf, S.A. ElShebiny, N.A. Salem, Neuroprotective effects of bee venom acupuncture therapy against rotenone-induced oxidative stress and apoptosis, *Neurochem. Int.* 80 (2015) 79–86.
- [62] S.S. Saini, J.W. Peterson, A.K. Chopra, Melittin binds to secretory phospholipase A 2 and inhibits its enzymatic activity, *Biochem. Biophys. Res. Commun.* 238 (1997) 436–442.
- [63] E.D. Mihelich, R.W. Schevitz, Structure-based design of a new class of anti-inflammatory drugs: secretory phospholipase A 2 inhibitors, SPI, *Biochimica et Biophysica Acta (BBA)-Mol. Cell Biol. Lipids* 1441 (1999) 223–228.
- [64] T. Lawrence, The nuclear factor NF- κ B pathway in inflammation, *Cold Spring Harbor Perspect. Biol.* 1 (2009) a001651.
- [65] D. Blum, S. Torch, N. Lambeng, et al., Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease, *Prog. Neurobiol.* 65 (2001) 135–172.
- [66] H. Mochizuki, K. Goto, H. Mori, Y. Mizuno, Histochemical detection of apoptosis in Parkinson's disease, *J. Neurol. Sci.* 137 (1996) 120–123.
- [67] W.G. Tatton, R. Chalmers-Redman, D. Brown, N. Tatton, Apoptosis in Parkinson's disease: signals for neuronal degradation, *Ann. Neurol.* 53 (2003) S61–S72.
- [68] Wu Y Le DA, Z. Huang, et al., Caspase activation and neuroprotection in caspase-3-deficient mice after in vivo cerebral ischemia and in vitro oxygen glucose deprivation, *Proc. Natl. Acad. Sci.* 99 (2002) 15188–15193.
- [69] A.-R. Doo, S.-N. Kim, S.-T. Kim, et al., Bee venom protects SH-SY5Y human neuroblastoma cells from 1-methyl-4-phenylpyridinium-induced apoptotic cell death, *Brain Res.* 1429 (2012) 106–115.
- [70] E.M. Halvorsen, J. Dennis, P.M. Keeney, et al., Methylpyridinium (MPP⁺)-and nerve growth factor-induced changes in pro-and anti-apoptotic signaling pathways in SH-SY5Y neuroblastoma cells, *Brain Res.* 952 (2002) 98–110.
- [71] A.-R. Doo, S.-T. Kim, S.-N. Kim, et al., Neuroprotective effects of bee venom pharmaceutical acupuncture in acute 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced mouse model of Parkinson's disease, *Neurol. Res.* 32 (2010) 88–91.
- [72] T. Kihara, S. Shimohama, H. Sawada, et al., Protective effect of dopamine D2 agonists in cortical neurons via the phosphatidylinositol 3 kinase cascade, *J. Neurosci. Res.* 70 (2002) 274–282.
- [73] K. Abe, Y. Abe, H. Saito, Agmatine induces glutamate release and cell death in cultured rat cerebellar granule neurons, *Brain Res.* 990 (2003) 165–171.
- [74] H. Kawasaki, T. Morooka, S. Shimohama, et al., Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells, *J. Biol. Chem.* 272 (1997) 18518–18521.
- [75] V. Chaparro-Huerta, M.C. Rivera-Cervantes, M.E. Flores-Soto, et al., Proinflammatory cytokines and apoptosis following glutamate-induced excitotoxicity mediated by p38 MAPK in the hippocampus of neonatal rats, *J. Neuroimmunol.* 165 (2005) 53–62.
- [76] S.M. Lee, E.J. Yang, S.-M. Choi, et al., Effects of bee venom on glutamate-induced toxicity in neuronal and glial cells, *Evid.-Based Complem. Altern. Med.* 2012 (2011).
- [77] A. Cristian, M. Katz, E. Cutrone, R.H. Walker, Evaluation of acupuncture in the treatment of Parkinson's disease: a double-blind pilot study, *Mov. Disord.* 20 (2005) 1185–1188.
- [78] L.M. Shulman, X. Wen, W.J. Weiner, et al., Acupuncture therapy for the symptoms of Parkinson's disease, *Mov. Disord.* 17 (2002) 799–802.
- [79] S.A. Thomas, R.D. Palmiter, Disruption of the dopamine B-hydroxylase gene in mice suggests roles for norepinephrine in motor function, learning, and memory, *Behav. Neurosci.* 111 (1997) 579.
- [80] D. Alvarez-Fischer, C. Noelker, F. Vulinović, et al., Bee venom and its component apamin as neuroprotective agents in a Parkinson disease mouse model, *PLoS One* 8 (2013) e61700.
- [81] N. Maurice, T. Deltheil, C. Melon, et al., Bee venom alleviates motor deficits and modulates the transfer of cortical information through the basal ganglia in rat models of parkinson's disease, *PLoS One* 10 (2015) e0142838.
- [82] B. Salthun-Lassalle, E.C. Hirsch, J. Wolfart, et al., Rescue of mesencephalic dopaminergic neurons in culture by low-level stimulation of voltage-gated sodium channels, *J. Neurosci.* 24 (2004) 5922–5930.
- [83] J.-I. Kim, E.J. Yang, M.S. Lee, et al., Bee venom reduces neuroinflammation in the MPTP-induced model of Parkinson's disease, *Int. J. Neurosci.* 121 (2011) 209–217.
- [84] S.-Y. Cho, S.-R. Shim, H.Y. Rhee, et al., Effectiveness of acupuncture and bee venom acupuncture in idiopathic Parkinson's disease, *Parkinsonism Related Dis.* 18 (2012) 948–952.
- [85] K.-H. Doo, J.-H. Lee, S.-Y. Cho, et al., A prospective open-label study of combined treatment for idiopathic parkinson's disease using acupuncture and bee venom acupuncture as an adjunctive treatment, *J. Altern. Complem. Med.* 21 (2015) 598–603, doi:http://dx.doi.org/10.1089/acm.2015.0078.
- [86] A. Hartmann, J. Müllner, N. Meier, et al., Bee venom for the treatment of parkinson disease –A randomized controlled clinical trial, *PLoS One* 11 (2016) e0158235, doi:http://dx.doi.org/10.1371/journal.pone.0158235.
- [87] A.I. Abushouk, A. Negida, H. Ahmed, M.M. Abdel-Daim, Neuroprotective mechanisms of plant extracts against MPTP induced neurotoxicity: future applications in Parkinson's disease, *Biomed. Pharmacother.* 85 (2016) 635–654.
- [88] A.I. Abushouk, N.M. Duc, Curing neurophobia in medical schools: evidence-based strategies, *Med. Educ. Online* 21 (2016) 32476.