Neuronal Cell Death and Mouse (Mus musculus) Behaviour Induced by Bee Venom

1Rian Oktiansyah, 1Berry Juliandi, 1Kanthi Arum Widayati and 2Vetnizah Juniantito

1Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor 16680, Indonesia
2Department of Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16680, Indonesia

Published online: 6 July 2018
To cite this article: Rian Oktiansyah, Berry Juliandi, Kanthi Arum Widayati and Vetnizah Juniantito. (2018). Neuronal cell death and mouse (Mus musculus) behaviour induced by bee venom. Tropical Life Sciences Research 29(2): 1–11. https://doi.org/10.21315/tlsr2018.29.2.1
To link to this article: https://doi.org/10.21315/tlsr2018.29.2.1

Abstract: Neuronal cell death can occur in a tissue or organ, including the brain, which affects memory. The objectives of this study were to determine the dose of bee venom that causes neuronal death and analyse the alteration of mouse behaviour, focusing in particular on spatial memory. Fifteen male mice of Deutsche Denken Yoken (DDY) strain were divided into control and treatment groups. Bee venom was injected six times for two weeks intraperitoneally with 1.88 mg/kg, 3.76 mg/kg, 5.6 mg/kg, and 7.48 mg/kg doses of venom. Brain histology was studied using haematoxylin-eosin stained paraffin embedded 5 µm coronal sections. A Y maze test was used to assay behaviour. Parameters observed were the number of dead neurons and the percentage of mice with altered behaviour. ANOVA showed that the effects of bee venom were significantly different in the case of the neuronal death parameter but were not significantly different in the case of the mice behaviour parameter. Duncan’s Multiple Range Test (DMRT) demonstrated that P4 (7.48 mg/kg) gave the highest effect of bee venom to promote neuronal death.

Keywords: Cell Death, Mice Behaviour, Bee Venom

INTRODUCTION

Cell death in multicellular organisms plays an important role during development. Cell death controls the number of cells and protects the organism by removing all cells damaged by disease, ageing, infections, genetic mutations, and exposure to toxic substances. Cell death in nature is categorised into two types: necrosis and apoptosis (Saikumar & Venkatachalam 2009).

Necrosis and apoptosis in response to external factors such as toxic substances and allergens are commonly induced responses (Cudrici et al. 2006). Morphologically, necrosis is indicated by shrinkage, fragmentation, or fusing the
nucleus of the cells. In contrast to necrosis, apoptosis is characterised by the presence of blebs on the plasma membrane and separation of cytoplasm and organelles of cells (apoptotic body) (Majno & Joris 1995). Necrosis and apoptosis can occur in the same tissue or organ, including in the hippocampus that affects memory.

Memory has an important function for humans, particularly related to intellectual performance and human behaviour. Memory is used for repeating previously performed activities and information retrieval, such as time, directions, purposes, or other important data (Langley 2012; Banikowski 1999). There is a product alleged to affect memory that it is not reported yet scientifically: bee venom. Bee venom is used as an alternative medicine as well as for the prevention of various diseases, such as arthritis, rheumatism, pain, cancer and neurodegenerative diseases (Kwon et al. 2001; Yang et al. 2010; Worlitzer et al. 2012; Bellik 2015). Bee venom contains a complex mixture of compounds, including polypeptides, enzymes, lipids, and amino acids. Some of these contents provide anti-inflammatory and anti-cell damage effects (Ovcharov et al. 1976; Ownby et al. 1997; Truskinovsky et al. 2001; Raghuraman & Chattopadhyay 2006; Ali 2012; Abdu & Alahmari 2013; Bogdanov 2015; Eze et al. 2016; Lee & Bae 2016). Bee venom is produced by two glands in a worker bees sting. Bee venom production increases during the first week of an adult bee worker life and reaches a maximum amount when the worker bee is involved in nest defence and forage. However, bee venom production decreases with age (Krell 1996). There are several studies demonstrating that bee venom can cause cell death.

Bee venom induces cell death in human lymphocytes (Gajski & Garaj-Vrhovac 2011). Bee venom also contains Phospholipase A, which has been shown to induce cell death by inactivating p38 MAPK (Jeong et al. 2011). Associated with bee venom’s mode of action as an allergen, there are few studies on allergens resulting in cell death, such as NFAT (Nuclear Factor of Activated T Cell), which triggers inflammation in the skin and can cause cell death (Kwon et al. 2016). Mice were injected with 2,4-dinitrofluorobenzen, which induces keratinocyte apoptosis due to the expression of First Apoptosis Signal (FAS) (Hedrych-Ozimina et al. 2011). Nevertheless, bee venom has not been reported yet scientifically to cause neuronal cell death, affecting the behaviour of mice. The objectives of this study are to determine the dose of bee venom that causes neuronal cell death and analyse the alteration of the behaviour of mice, particularly spatial memory.

**MATERIALS AND METHOD**

**Time and Place**

The study was conducted at the Division of Biological Function and Animal Behaviour, Biology Department, Faculty of Mathematics and Natural Sciences and Pathology Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University. The study conducted from October 2015 until March 2016.
Research Animal

Mice used in this study were three-month old male Deutsche Denken Yoken (DDY) mice, approximately 20–30 g in weight. Mice were obtained from the Non-Ruminant Laboratory and Animals Prospect, Faculty of Animal Sciences, Bogor Agricultural University.

Experimental Unit

Fifteen mice used in this study were divided into control and treatments groups. Each treatment group consisted of three mice. Mice were reared in different cages, according to the group.

The Treatments of Animals

This study was approved by the ethics committee of Bogor Agricultural University (No. 6-2016 RSHP FKH-IPB). Mice were acclimatised for a week before the treatment. Mice were maintained in the laboratory at room temperature with wood chips. Mice were given water ad libitum throughout the study (Clark et al. 2008). Bee venom was injected intraperitoneally after acclimatisation. Bee venom was injected for two weeks (days 1, 4, 7, 10, 13, and 16) with the following doses: 1.88 mg/kg, 3.76 mg/kg, 5.6 mg/kg, and 7.48 mg/kg, respectively (Bogdanov 2015). The bee venom doses used in this study were varied from a minimal dose, an optimal dose, and an exceeds optimal dose to allow study of its effect on cell death. The highest dose selected was considered high enough to cause cell death. It was possible to see a dose dependency of neuronal cell death at higher concentrations. However, the doses administered were sufficient to observe the effect of bee venom on neuronal cell death. Bee venom was obtained from an apitherapy clinic. Morphological examination of brain tissue was performed using sectioned tissue, stained with haematoxylin-eosin (HE). Behaviour test was performed after injection. The purpose of the behaviour test is to examine the spatial memory of mice using a Y-maze (Onaolapo et al. 2012). Behaviour tests were performed between 1.30 to 4.30 pm (Juliandi et al. 2015). This study is designed as preliminary screening study. This research is expected to provide early information allowing further research using additional behaviour tests and to determine optimal doses of bee venom. Brain tissue isolation was performed three days after the last injection of bee venom using the perfusion method (Matsuda 2007; Erwin et al. 2013).

Experimental Design and Data Analysis

This study used a completely randomised design method that consisted of five treatments and three repetitions. The parameters observed were the percentage of mice to explore the Y maze correctly and the number of dead neurons. Data were analysed using analyses of variance (ANOVA).
RESULTS

Mice Brain (Dentate Gyrus) Histology

Based on observation on histological sections, there was neuronal cell death in the dentate gyrus (Fig. 2). The results showed differences in the average number of dead neuronal cells in each dentate gyrus section. The average number of dead neuronal cells fluctuated in each treatment compared to control. The highest average of dead neurons was 1510 cell/mm², and the lowest average was 220 cell/mm². The fluctuation of the average number of dead cells demonstrated that bee venom can lead to neuronal cell death in the dentate gyrus. The fluctuation of these averages can be seen in Fig. 1.

![Figure 1: Mean of neuronal cells death in dentate gyrus after 2 weeks of bee venom injections with the following doses P1 = 1.88 mg/kg, P2 = 3.76 mg/kg, P3 = 5.6 mg/kg, and P4 = 7.48 mg/kg](image)

Fig. 2 demonstrated that neuronal cells death in the dentate gyrus. Dead neurons were marked by cells with dark staining and the absence of a nucleus. ANOVA showed that the effect of bee venom lead to a significantly different number of dead neuronal cells in the dentate gyrus. This suggests that bee venom has a role in the regulation of neuronal cell death in the dentate gyrus. Thus, the Duncan’s Multiple Range Test (DMRT) was required to further examine the effect of treatments on the regulation neuronal cell death in the dentate gyrus.

The DMRT results demonstrated that P4 was significantly different from other treatments. Treatment with this highest dose of bee venom led to the highest number of neuronal cell deaths in the dentate gyrus. Based on this study, many doses of bee venom gave the significant effect on neuronal cells death. Furthermore, the effect of bee venom on neuronal cell death was significant using P4 (7.48 mg/kg).
Figure 2: Representative image of brain sections containing dentate gyrus in each treatment stained by HE. Bee venom doses P1 = 1.88 mg/kg, P2= 3.76 mg/kg, P3=5.6 mg/kg, and P4=7.48 mg/kg. Dead neurons are shown by arrow (bars: 50 μm)
Mice Behaviour

The movement of mice through the Y-maze arm was tracked in this study. The results displayed differences in the average percentage of alteration in mice behaviour. The percentage of alteration in mice behaviour in the Y-maze fluctuated in each treatment compared to control. The highest average of alteration after 24 h bee venom injection was 74.57%, and the lowest was 61.07%. The highest average of alteration after 72 h bee venom injection was 76.03%, and the lowest was 57.50%. The fluctuation of the average showed that bee venom has an influence on altering the behaviour of mice in the Y-maze. This fluctuation can be seen in Fig. 3.

Analysis of variance (ANOVA) showed that the effect of bee venom was not significantly different between mice treatments. Thus, bee venom has no influence on mice behaviour. Although bee venom did not significantly alter mice behaviour, the percentage of alteration mice behaviour in the Y-maze, in addition to the number of neuronal cell deaths in the dentate gyrus indicates that bee venom has an effect.

DISCUSSION

Neuronal Cell Death in the Dentate Gyrus

Based on this study, bee venom affects neuronal cell death in the dentate gyrus. The DMRT test showed that the highest dose of bee venom was significantly different from other treatments on neuronal cell death in the dentate gyrus. A dose of 4 mg/kg body weight in rats is the maximum dose of bee venom required to stimulate an anti-inflammatory effect, while the higher dose shows neurotoxic effects (Bogdanov 2015). Fig. 2 demonstrates that neuronal cell deaths can be found in the subgranular zone (SGZ). According to the study, we proposed that the dead neurons are neural progenitor cells (NPC) because they were found in the innermost layer of SGZ. However, special markers would be needed to confirm these cell types.

The increase in the number of neuronal cell deaths in the dentate gyrus after P4 treatment suggested that there may be an influence of inflammatory compounds in the bee venom. Previous studies have demonstrated the role of melittin, phospholipase A2, apamin, and adolapin in high doses contained in bee venom to cause allergies, lysis of erythrocytes, myonecrosis, and other neurotoxic effects (Ovcharov et al. 1976; Ownby et al. 1997; Raghuraman & Chattopadhyay 2006; Ali 2012; Abdu & Alahmari 2013; Elhakim et al. 2014; Bogdanov 2015; Eze et al. 2016; Lee & Bae 2016). Therefore, bee venom can affect the peripheral tissues.

The influence of bee venom in peripheral tissues could be expected to produce cytokines that signal the brain, causing neuronal cell death in the dentate
gyrus. Dantzer et al. (2008), Dilger and Johnson (2008), and Maier and Linda (2012) demonstrated that cytokines from peripheral tissues transported to the brain via the endocrine system can induce neuronal cell death. Palombella and Vilcek (1989) reported that melittin and phospholipase A2 can activate cytokines, in particular TNF. Although this still needs further confirmation, the administration of bee venom is also supposed to indirectly effect neuronal cell death in the dentate gyrus via cytokine signalling.

Another suggestion about the mechanism of neuronal cell death induced to bee venom, is the ability of bee venom compounds to pass through the blood brain barrier (BBB). The BBB plays an important role in regulating the molecules that can pass into and out of the brain (Ransohoff & Engelhardt 2012; Takeshita & Ransohoff 2012). Oller-Salvia et al. (2013) and Mourre et al. (1997) reported that apamin, a compound of bee venom, can pass through the blood brain barrier and cause neuronal cell death. Other compounds of bee venom, however, have not been reported yet to pass through the BBB. Therefore, apamin could be the causative agent of neuronal cell death in the dentate gyrus.

There was no linear relationship on neuronal cell death in the dentate gyrus with an increasing dose of bee venom. The higher doses of bee venom did not lead higher numbers of neuronal cell deaths in the dentate gyrus. In this study, P2 showed a higher number of dead neuronal cells compared to control, P1, and P3. This anomalous result of P2 does not affect the conclusions from this study. This may be due to the efficiency of bee venom utilisation in P1 treatments. P1 had the lowest amount of neuronal cell death in the dentate gyrus compared to the other treatments. These results provide straightforward information about the efficiency of administration for the apitherapy clinic and the people who want to utilise bee sting therapy.

The Spatial Learning Ability of Mice

The spatial learning ability of mice is shown by the correct-arm alternation in Y-maze. The Y-maze was first used to examine the spatial learning underlying mice exploring a new environment. Wolfe (1969) reported that mice prefer to investigate new objects and environments compared with known objects and environments. This study showed that bee venom administration tended to effect the spatial learning ability of mice (Fig. 3). The tendency of these effects can be caused by neuronal cell death in the dentate gyrus. The dentate gyrus plays a role in memory formation, in particular related to cognitive function and spatial memory (Silva et al. 1998; Eichenbaum et al. 1999). Our results demonstrated that the treatment that induced the most dead neurons had the lowest correct-arm alternation in the Y-maze (Fig. 3). We hypothesize that NPCs made up the bulk of dead neurons in the SGZ. Dead or damaged NPCs will cause mature neurons to be reduced in size while the remaining mature neurons will undergo programmed cell death. This will affect the process of learning and spatial memory in mice.
The ANOVA test showed that the number of neuronal cell deaths was not significantly different with respect to altered mice behaviour, particularly spatial memory. The number of neuronal cell deaths in the dentate gyrus induced by bee venom was still under the threshold level, so it could not affect mice behaviour yet. This is consistent with Conrad and Roy (1993), who reported that as much as 80% of neuronal cell death in the dentate gyrus does not affect spatial memory. Although the ANOVA test showed that there was no significant difference, there was a downward trend in the percentage of correct-arm alternation in the Y-maze.

CONCLUSION

Based on the study, bee venom has neurotoxic effect because it caused neuronal cell death in the dentate gyrus and behaviour alteration in mice. The dose of bee venom that caused the highest neuronal cell deaths in the dentate gyrus was P4 (7.48 mg/kg). The percentage of mice with altered behaviour in a Y-maze test along with the number of neuronal cells death in the dentate gyrus corroborates this.

ACKNOWLEDGEMENTS

We thank Mr. Tyanto for providing bee venom solution, the biology team (Arif Mohammad Siddiq, Quratul Edritanti, Anggun Wicaksono, Aurora Fathyaa, Winati Nurhayu, Laurentia Henrieta Perimita Sari Purba, Jeane Siswitasari Mulyana,
and Dian Rahma Pratiwi) for assistance in the laboratory, and LPDP for financial support. We also thank Ministry of Research, Technology and Higher Education, Indonesia for providing research grant No. 011/SP2H/LT/DRPM/IV/2017 to Berry Juliandi.

REFERENCES

Abdu F and Alahmari A. (2013). Anti-inflammatory effect of melittin on mice jejunum. *Global Advanced Research Journal of Environmental Science and Toxicology* 2(3): 68–76.

Ali M A A M. (2012). Studies on bee venom and its medical uses. *International Journal of Advancements in Research & Technology* 1(1): 1–15.

Banikowski A K. (1999). Strategies to enhance memory based on brain-research. *Educational Psychology* 32(2): 1–22.

Bellik Y. (2015). Bee venom: its potential use in alternative medicine. *Anti-Infective Agents* 13(1): 1–14. https://doi.org/10.2174/2211352513666150318234624

Bogdanov S. (2015). Bee Venom: Composition, Health, Medicine: A Review. *Bee Product Science* 1(1): 1–20.

Clark P J, Brzezinska W J, Thomas M W, Rhyzenko NA, Toshkov SA and Rhodes J S. (2008). Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in c57bl/6j mice. *Neuroscience* 155(1): 1048–1058. https://doi.org/10.1016/j.neuroscience.2008.06.051

Conrad C D and Roy E J. (1993). Selective loss of hippocampal granule cell following adrenalectomy: implications for spatial memory. *Journal of Neuroscience* 13(6): 2582–2590.

Cudrici C, Niculescu T, Niculescu F, Shin M L and Rus H. (2006). Oligodendrocyte cell death in pathogenesis of multiple sclerosis: protection of oligodendrocytes from apoptosis by complement. *Journal of Rehabilitation Research & Development* 43(1): 123–132. https://doi.org/10.1682/JRRD.2004.08.0111

Dantzer R, O’Connor J C, Freund G G, Johnson R W and Kelley K W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience* 9(1): 46–56. https://doi.org/10.1038/nrn2297

Dilger R N and Johnson R W. (2008). Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. *Journal of Leukocyte Biology* 84(1): 932–939. https://doi.org/10.1189/jlb.0208108

Eichenbaum H, Dudchenko P, Wood E, Shapiro M and Tanila H. (1999). The hipokampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron* 23: 209–226. https://doi.org/10.1016/S0896-6273(00)80773-4

Elhakim Y M A, Khalil S R, Awad A and Ayadhi L Y A. (2014). Combined cytogenotoxic effect of bee venom and bleomycin on rat lymphocytes: An in vitro study. *BioMed Research International* 1(1): 1–9. https://doi.org/10.1155/2014/173903

Erwin, Pangestiningsih T W and Widyarini S. (2013). Kepadatan sel hipokampus insulin imunoreaktiv pada formasi hipokampus mencit yang diinduksi berulang dengan streptozotosin. *Jurnal Veteriner* 14(2): 126–131.

Eze O B L, Nwodo O F C and Ogugua V N. 2016. Therapeutic effect of honey bee venom. *Journal of Pharmaceutical, Chemical, and Biological Sciences* 4(1): 48–53.
Gajski G and Garaj-Vrhovac V. (2011). Bee venom induced cytogenetic damage and decreased cell viability in human white blood cells after treatment in vitro: A multi-biomarker approach. *Environmental Toxicology and Pharmacology* 32(1): 201–211. https://doi.org/10.1016/j.etap.2011.05.004

Hedrich-Ozimina A, Behrendt K, Hao Z, Pofahl R, Ussath D, Knaup R, Krieg T and Haase I. (2011). Enhanced contact allergen- and UVB-induced keratinocyte apoptosis in the absence of CD95/Fas/Apo-1. *Cell Death and Differentiation* 18(1): 155–163. https://doi.org/10.1038/cdd.2010.83

Jeong J K, Moon M H, Bae B C, Lee Y J, Seol J W and Park S Y. (2011). Bee venom phospholipase A2 prevents prion peptide induced-cell death in neuronal cells. *International Journal of Molecular Medicine* 28(1): 867–873.

Juliandi B, Tanemura K, Igarashi K, Tominaga T, Furukawa Y, Otsuka M, Moriyama N, Ikegami D, Abematsu M, Sanosaka T et al. (2015). Reduced adult hippocampal neurogenesis and cognitive impairments following prenatal treatment of the antiepileptic drug valproic acid. *Stem Cell Reports* 5(1): 1–14.

Krell R. (1996). Value-added products from beekeeping. *FAO Agricultural Services Bulletin* 124(1): 1–13.

Kwon H K, Kim G C, Hwang J S, Kim Y, Chae C S, Nam J H, Jun C D, Rudra D, Surh C D and Im S H. (2016). Transcription factor NFAT1 controls allergic contact hypersensitivity through regulation of activation induced cell death program. *Scientific Reports* 1(1): 1–15. https://doi.org/10.1038/srep19453

Kwon Y B, Lee J D, Lee H J, Han H J, Mar W C, Kang S K, Beitz A J and Lee J H. (2001). Bee venom injection into an acupuncture point reduces arthritis associated edema and nociceptive responses. *Pain* 90(1): 271–280. https://doi.org/10.1016/S0304-3959(00)00412-7

Langley P. (2012). Intelligent behaviour in humans and machines. *Advances in Cognitive Systems* 2(1): 3–12.

Lee G and Bae H. (2016). Bee venom phospholipase A2: Yesterday’s enemy becomes today’s friend. *Toxins* 8(48): 1–12. https://doi.org/10.3390/toxins8020048

Maier S F and Linda R. (2012). Consequences of the inflamed brain. *Dana Alliance* 1(1): 1–4.

Majno G and Joris I. (1995). Apoptosis, oncosis, and necrosis an overview of cell death. *American Journal of Pathology* 146(1): 3–15.

Matsuda H. (2007). Role of neuroimaging in Alzheimer’s disease, with emphasis on brain perfusion. *The Journal of Nuclear Medicine* 48(8): 1289–1300. https://doi.org/10.2967/jnumed.106.037218

Mourre C, Fournier C and Soumireu-Mourant B. (1997). Apamin, a blocker of the calcium-activated potassium channel, induces neurodegeneration of purkinje cells exclusively. *Brain Research* 778(1): 405–408. https://doi.org/10.1016/S0006-8993(97)01165-7

Oller-Salvia B, Teixido M and Giralt E. (2013). From venom to BBB shuttles: Synthesis and blood-brain barrier transport assessment of apamin and a nontoxic analog. *Biopolymers* 100(6): 675–686. https://doi.org/10.1002/bip.22257

Onaolapo O J, Onaolapoh A Y, Mosaku T J, Akanji O O and Abiodun O R. (2012). Elevated plus maze and Y-maze behavioural effects of subchronic, oral low dose monosodium glutamate in swiss albino mice. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 3(4): 21–27.

Ovcharov R, Shkenderov S and Mihailova S. (1976). Anti-inflammation effects of apamine. *Toxicon* 14(1): 441–447. https://doi.org/10.1016/0041-0101(76)90060-X
Ownby C L, Powell J R, Jiang M S and Fletcher J E. (1997). Melittin and phospholipase A2 from bee (Apis mellifera) venom cause necrosis of murine skeletal in vivo. *Toxicon* 35(1): 67–80.

Palombella V J and Vilcek J. (1989). Mitogenic and cytotoxic actions of tumor necrosis factor in BALB/c 3T3 cells. *The Journal of Biological Chemistry* 264(30): 18128–18136.

Raghuraman H and Chattopadhyay A. (2006). Melittin: a membrane-active peptide with diverse functions. *Bioscience Report* 27(1): 189–223.

Ransohoff R M and Engelhardt B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. *Nature Reviews Immunology* 12(9): 623–635. https://doi.org/10.1038/nri3265

Saikumar P and Venkatachalam M A. (2009). Apoptosis and cell death. *Molecular Pathology Library* 14(1): 29–41. https://doi.org/10.1007/978-0-387-89626-7_4

Silva A J, Giese K P, Fedorov N B, Frankland P W and Kogan J H. (1998). Molecular, cellular, and neuroanatomical substrates of place learning. *Neurobiology of Learning and Memory* 70(1): 44–61. https://doi.org/10.1006/nlme.1998.3837

Takeshita Y and Ransohoff R M. (2012). Inflammatory cell trafficking across the blood-brain barrier (BBB): Chemokine regulation and *in vitro* models. *Immunological Reviews* 248(1): 228–239. https://doi.org/10.1111/j.1600-065x.2012.01127.x

Truskinovsky A M, Dick J D and Hutchins G M. (2001). Fatal infection after a bee sting. *Clinical Infectious Diseases* 32(2): 36–38. https://doi.org/10.1086/318451

Wolfe J L. (1969). Exploratory activity and new object response of wild and laboratory house mice. *Communications in Behaviour Biology* 4(1): 13–16.

Worlitzer M M A, Bunk E C, Hemmer K and Schwamborn J C. (2012). Anti-inflammatory treatment induced regenerative oligodendrogenesis in parkinsonian mice. *Current Stem Cell Research and Therapy* 3(1): 33. https://doi.org/10.1186/scrt124

Yang E J, Jiang J H, Lee S M, Yang S C, Hwang H S, Lee M S and Choi S M. (2010). Bee venom attenuates neuroinflammatory events and extends survival in amyotrophic lateral sclerosis models. *Journal of Neuroinflammation* 7(1): 69. https://doi.org/10.1186/1742-2094-7-69