P-ISSN: 2618-0723
E-ISSN: 2618-0731

NAAS Rating: 5.04
www.extensionjournal.com

International Journal of Agriculture Extension and Social Development

Volume 7; SP-Issue 9; September 2024; Page No. 160-165

Received: 01-06-2024
Accepted: 08-07-2024

Indexed Journal
Peer Reviewed Journal

Bee venom: From pain to profit

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DOI: https://doi.org/10.33545/26180723.2024.v7.i9Sc.1100

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Abstract

Apitherapy is used as an alternative medicine practice of using bee products. Bee sting was used to cure arthritis in old days. Recent researches have shown that bee venom may help reduce autoimmune symptoms by decreasing inflammation and enhancing immunity. It has antimicrobial, anti-inflammatory, antioxidative, radioprotective, and regenerative properties. Bee venom therefore have a good demand in pharmaceutical industries for the development of medicines related to human and animal disease treatments. Venom can be collected from bees using devices that extract venom from bees without killing them. The global market for bee venom is likely to reach nearly US$ 540 million by 2032.There is no national legislation for bee venom production, or marketing. The organized market is almost non-existent. It is essential to create awareness in consumers as well as apiculturists regarding the future market for bee venom.

Keywords: Bee venom, apitherapy, medical properties, venom extraction, economic prospects

Introduction

For centuries, honeybees have been revered for their numerous benefits, which go beyond their iconic role as honey producers. The medicinal use of honey and other bee products is deeply rooted in history, with mentions of their curative properties found in sacred texts such as the Veda, Bible, and Quran. The origins of apitherapy, a form of therapy utilizing bee products, are obscure and multifaceted. Historical records indicate that apitherapy was practiced in ancient civilizations, including Egypt and Greece, with a documented history in China spanning 3,000 to 5,000 years [1]. Apitherapy is the art and science of using bee products *viz*. honey, pollen, bee bread, propolis, royal jelly, venom, etc. for human welfare [2]. Bee venom is odorless, colorless, clear watery liquid with ornamental pungent smell, a bitter taste and basic pH [3,4]. Bee uses it used for self-defense. Bee venom (BV) also referred to as apitoxin, is produced by sting glands present in the abdomen of worker honey bees. When females experience any danger, they inject venom through their sting glands [5]. Beyond the initial pain and swelling, honeybee venom has been discovered to hold remarkable medicinal value. By harnessing this venom's therapeutic potential, scientists have developed innovative treatments, showcasing the profound impact of these tiny insects on human health [6]. The medicinal use of bee venom apparently dates back to ancient Egypt and is reported in the histories of Europe and Asia. Hippocrates (460-370 BC) used bee venom to treat joint pain and arthritis. The Greek physician Galen (129-199 A.D.) mentioned the uses of bee venom for treating human diseases. Bee sting therapy for treating rheumatic diseases has been known for many years. Filip Terc (1844-1917), a doctor and beekeeper, cured more than 500 patients from rheumatic diseases using bee stings. He is referred to as the father of Apitherapy. Dr. Rudolph Tertsch, the father of modern apitherapy, treated more than 600 patients with bee stings. Dr. Bodog F. Beck specialized in the treatment of arthritic and rheumatoid conditions using bee venom. He coined the term "bee venom therapy" (BVT). The dried form is of yellow color. It is a bitter, odorless, colorless acidic liquid with a pH of 4.5-5.5 and contains a mixture of different active molecules such as peptides including melittin, apamin, adolapin, mast cell degranulating peptide, and enzymes phospholipase A2 (PLA2) and hyaluronidase [7]. Recent years have seen great interest in BVT as alternative medicine. Additionally, research on bee venom implies its applications for developing cures for ailments of humans and animals [8,9]. Bee venom exhibits a diverse range of pharmacological properties, including anti-inflammatory Antioxidant: neutralizing free radicals and protecting against cell damage, potentially treating neurological disorders, protecting against radiation-induced damage combating bacterial infections, antiviral and antifungal properties. These properties make bee venom a valuable ingredient in the development of various products, including cosmetics. Researchers and pharmaceutical companies are actively exploring bee venom (BV) to unlock its full potential in modern medicine.

Biochemical constituents of BV

Basically, BV is colorless, odorless liquid with 88% water. A drop of BV comprises only 0.1 g of dry venom [10]. It has acidic PH ranging in between 4.5-5.5. Biochemical composition of BV may slightly vary based on the species of bee, time of the year, season, locality etc. Its specific weight is 1.1331, indicating a dense, protein-rich composition. Enzymes (e.g., phospholipase A2, hyaluronidase), Peptides (e.g., melittin, apamin, Biogenic amines (e.g., histamine, dopamine) are the main constituents [11]. Melittin is the primary constituent of bee venom, accounting for approximately 50% of the weight of dried bee venom. This potent peptide is the main bioactive component responsible for the therapeutic properties of BV [12]. melittin is not only the dominant, but also the main lethal component in BV. The linear structure of this amphiphilic peptide contains 26 amino acids [13]. Melittin has comprehensive anti-inflammatory, hemolytic, anti-microbial, anti-fungal, and antitumor properties [14, 15]. Sulfur is believed to be the main element in BV inducing the release of cortisol from the adrenal glands and in protecting the body from infections.

Phospholipase is the most significant allergen and hence the most toxic element of bee venom. Phospholipase A_2 consists of 12% of venom It has promising anticancer, antiprotozoal, anti-inflammatory, immunomodulatory, anti-bacterial, anti-viral effects [16, 17]. Nine percent of BV consists of histamine. It expands blood vessels and promotes the permeability of capillaries [18]. Apamin makes up 3% of venom, destroys nerve tissue. Apamin shows anti-fungal, anti-viral, anti-inflammatory, analgesic activity. Apamin has been investigated for its therapeutic potential in various diseases, including ataxia, epilepsy, and inflammatory conditions. [19]. Hyaluronidase (2% of venom,) helps the reaction to spread to surrounding tissue by breaking down one of the components of cell tissue [20]. Dopamin has anti-arthritic, analgesic, anti-inflammatory, antipyretic, anti-nociceptive effects [21].

The composition of venom may differ among different species or even among variants of the same bee species. Higher level of PLA2 was found in the venom of the *A. mellifera scutellata* than in the *A. mellifera mellifera* [22, 23]. Melittin content was found to present in 59.3% of total venom content in Asian honey bee venom [24]. The melittin content in *A. dorsata, A. mellifera, A. florea, and A. cerena* were 95.8 ± 3.2%, 76.5 ± 1.9%, 66.3 ± 8.6%, and 56.8 ± 1.8%, respectively [25].

BV for human diseases treatments

The lethal dose of bee venom for an adult human is estimated to be approximately 2.8 milligrams per kilogram of body weight. Given that a single bee contains only 0.15-0.30 milligrams of venom, it would require a large number of stings to deliver a fatal dose [26]. The crude extracts of BV from *Apis cerana, A. dorsata, A. florea* have antimicrobial effects against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli, Xanthomonas subtilis, Proteus vulgaris, Salmonella typhimurium* and *Candida albicans* [25]. BV has been shown to have analgesic, anti-cancer, anti-asthmatic, antioxidant, anti-aging, anti-atherosclerotic, anti-diabetic, hepatoprotective, antiviral, neuroprotective and anti-rheumatoid arthritis biological activities**.** Especially, melittin has attracted a lot of attention in recent years for its anti-cancer activity. Numerous research studies have examined the impact of melittin's regulation of apoptosis and the factors that cause apoptosis in different cancer types, including breast, stomach, kidney, ovarian, prostate, gastric, colon, esophageal, and lung and cervical cancer [27-30]. Melittin indues apoptosis, cell cycle arrest, modulation oncogenic signaling pathway, inhibiting metastasis, and promoting chemo/radio sensitivity. In several research studies, the melittin component of bee venom has shown significant inhibition of cell proliferation of several human cancer cells via stimulation of lymph nodes associated with local cellular immune response. The mechanism behind this bee venom mediated growth inhibition includes apoptosis, lysis, and necrosis. Additionally, melittin also possesses antimutagenic, anti-inflammatory, radioprotective, and anti-nociceptive activities. It also showed anti-tumor efficacy towards pancreatic cancer cell lines [29]. BV is also found to enhance the efficiency of tamoxifen, a medication used for breast cancer to manage the disease.

BV and melittin are of significant potential in the treatment of some neurodegenerative diseases, including Parkinson's disease [31,32], Alzheimer's disease [33,34] and autoimmune diseases (such as rheumatoid arthritis [35], lupus erythematosus [36], and multiple sclerosis [37]. Bee venom administration has protective effects against the development of hepatic encephalopathy, a serious neurodegenerative disease [38].

BV has tremendous potential in dermatology and can serve as a more cost-effective, less irritating, readily available, and natural alternative to other medications. Various studies have revealed its therapeutic applications for the treatment of skin diseases like *Acne vulgaris*, atopic dermatitis, unhealed leg ulcers, diabetic foot ulcers, selected bacterial and viral infections, photoaging, and psoriasis [39-41]. It has shown promising results against migraine, epilepsy, malaria, neuralgia, chronic pain, nucroses, polyneuritis, asthama, decreased blood cholesterol level, soar throat, endoarthritis, etc [19, 42].

BV for Animal disease treatments

BV has been used to prevent and treat bovine mastitis as natural antimicrobial compounds in some dairy cattle farms in Korea. a dose of 12 mg of bee venom and a treatment period of more than 7 days were required to treat mastitis [43]. Experimental results support the hypothesis that BV can decrease LPS-induced inflammatory responses in bovine mammary epithelial cells through inhibition of oxidative stress [44]. BV injections at acupoints are more effective at protecting canines from intervertebral disc diseases [45]. The average activity of the arthritic dogs resembled that of the healthy dog population after just three doses of BV [46]. According to researchers, some canine arthritic disorders may be greatly improved by bee venom treatment. In horse BV is effective in treating arthritis, laminitis, chronic obstructive pulmonary disease. BV impact lymphocyte proliferation and viability in dogs, horses, and chickens. More research both *in vitro* and *in vivo* will be necessary to draw conclusions regarding their future use as immune stimulants or inhibitors [47]. The already numerous *in vitro* studies must be followed by clinical trials to determine a conscious and effective use of bee venom in veterinary clinical practice [48]

Extraction of BV

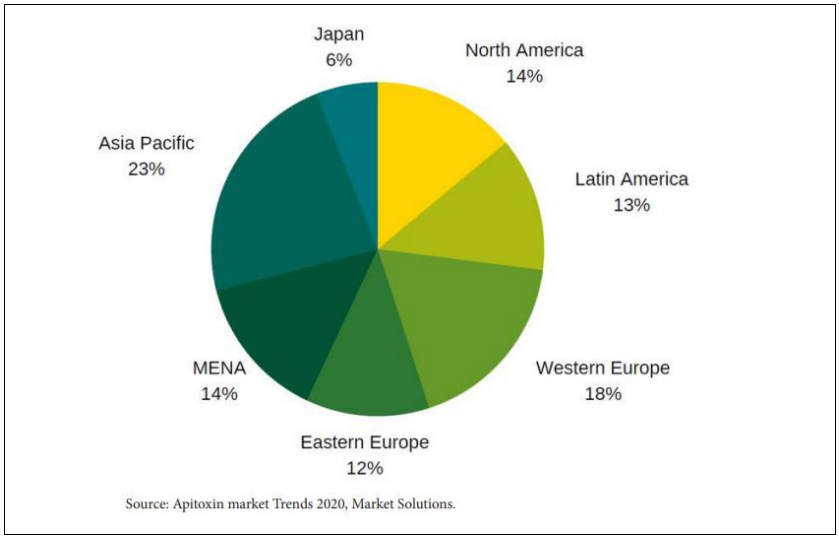
A device that provides an electric shock makes it possible to collect pure venom from several thousand honey bees [49]. A glass plate is kept outside or inside the hive. There are several different pieces of equipment on the market that are used both inside and outside the beehives [50]. When bee come in contact with the plate, gets the electric shock and releases venom as its natural defense mechanism. Typically, the voltage is between 24 and 30 volts, the pulse lasts between 2 and 3 s, there is a 3 to 6 s gap, and the pulse frequency is between 50 and 1000 hertz. Exterior collectors, placed outside the beehive, have limited exposure to bees, affecting only those entering and leaving the hive. This results in minimal bee venom production [51,52]. However, in this type of equipment, weather conditions and contaminants can compromise the venom's quality. Rainfall renders the equipment unusable. Venom exposure to environmental factors can lead to degradation or spoilage [53,54]. Internal collectors, placed inside the beehive, offer better venom collection due to increased bee contact. However, base collectors risk contamination from bee waste. Cover collectors have restricted collection areas due to beehive size [51,55]. Although in terms of investments, internal collector system is twice more expensive than the traditional one, significantly higher yield and better quality of bee venom shortens the return of investment significantly. There is a need to experiment and explore the area of venom extraction and come up with standard equipment [56,57]

Standardization of BV

Although there is no international available standard, certain criteria should be satisfied when bee venom is used within the technological production processes in the pharmaceutical industry [58,59]. Water content to be less than 2%, water insoluble substances up to 0.8%, sugars up to 6.5% and satisfying biological activity of hyaluronidase, phospholipase, melitin and protease inhibitors [60] The LD50 for bee venom is 3.7 milligrams per kilogram of body weight [61]. As on today, bee venom is not recognized as food or drug, there is no official quality standards set for it [48]. Guralnick (1986) described standardization and quality control of hymenoptera venom, including that of honeybee [62]

Market potential and economic prospects of BV

In India, There is no national legislation for bee venom production, or marketing. The organized market is almost non-existent, and consumers are mainly pharma industries both in medical and cosmetical areas. Global bee venom market and price are steadily growing by single digit numbers dominated by few big international players. Study published by Future market in 2022, states that the global market for bee venom extract is estimated at US$ 327.5 million. The market is likely to reach nearly US$ 540 Million by 2032, with a growing CAGR of 5.1% from 2022 to 2032 [63]. The most of the bee venom is sold by direct placement on the market (30%) and 27% through intermediary production contracts, of which 14% are without a pre-production contract. A small percentage of sales is realized through bee associations, 5%, and less than that, only 4% are intermediary contracts with a pre-production contract. Only 2% of sales are through friends and acquaintances, and classic export sales are only 1%. Therefore, one of the primary focus of producers is to work on development of distribution channels and faster "production to market" access. Depending whether is for cosmetic or for medical purposes, future market supply and demand will heavily depend on the profound and extensive research activities, innovation and modernization of production of the bee venom. Market needs to develop with greater dynamics. The absence of generally established standards for classification, quality and application, as well as the low level of research that would increase applicability, limit the production and sale of bee venom. The potential is great, but the need has to be generated, created and developed in several directions, from new studies and areas of application, to modernization of production, innovation, standardization and opening of new markets and sales channels. The primary priority is the adoption of a regulation that will standardize licensing, production processes, quality control, and professional staff. In this way, the production will be better planned, the method of collection will be improved, the technology will become more accessible, the storage, the method of keeping and the transportation of the collected bee venom before it goes to the production facilities that would further include it into cosmetic grade or medical grade formulations (64).



Source: Apitoxin market Trends 2020, Market Solutions.

In India, Usually, farmer struggle to sell the bee venom and the main difficulty they face is they don't have another option to sell venom apart from pharmaceutical companies. These companies does not show interest in purchasing small quantities of BV like 50-60 gram and they demand for BV in kg. producing 1 kg venom is very costly for farmers because they need bee hive boxes around 1600 - 2000 and much more venom collector machines nearly 800; the price of one venom collector is around Rs 10,000 to 11,000 so it makes a huge difference and it becomes almost impossible for small farmer to invest such a big amount.

Conclusion

The bee venom industry's rise highlights a harmonious balance between economic opportunity and ecological responsibility, as sustainable practices are employed to meet the surging demand for this unconventional yet valuable resource.

Although mechanisms of action and many bioactive substances still remains unclear. However, researchers believe that the ongoing work on this aspect will ultimately allow bee venom and its active components to be considered in the coming years as definitive candidates in various treatments.

It is necessary to significantly increase the funding of new research and to confirm and expand certain therapeutic areas of action. Efforts are needed to raise knowledge, awareness, modernize the bee venom collection process and build capacities for local production of specialized preparations for cosmetic and medical use. The creation of final products at the local level will allow the benefits to be directly felt by individual producers, national economies and through synergies to realize a wider economic and medical benefit.

References

1. Rose A. Bees in balance. Bethesda, Maryland: Starboint Enterprises, Ltd; c1994.
2. Fratellone PM, Tsimis F, Fratellone G. Apitherapy products for medicinal use. J Altern Complement Med. 2016 Dec;22(12):1020-2. DOI: 10.1089/acm.2015.0346. Epub 2016 Jul 27. PMID: 27463767.
3. Krell R. Value-added products from beekeeping. Rome, Italy: Food and Agriculture Organization; c1996. ISBN 9251038198.
4. Schmidt JO. Toxicology of venoms from the honeybee genus Apis. Toxicon. 1995;33(7):917-927.
5. Abdela N, Jilo K. Bee venom and its therapeutic values: A review. Adv Life Sci Technol. 2016;44:18-22.
6. Sadek KM, Shib NA, Taher ES, Rashed F, Shukry M, Atia GA, *et al*. Harnessing the power of bee venom for therapeutic and regenerative medical applications: an updated review. Front Pharmacol. 2024;15:1412245. DOI: 10.3389/fphar.2024.1412245.
7. Bellik Y. Bee venom: Its potential use in alternative medicine. Anti-Infective Agents in Medicinal Chemistry. 2015;13:3-16. DOI:10.2174/2211352513666150318234624.
8. Kwon Y, Lee J, Lee H, Han H, Mar W, Kang S, *et al*. Bee venom injection into an acupuncture point reduces arthritis-associated edema and nociceptive responses. Pain. 2001;90:271-280. DOI: 10.1016/S0304-3959(00)00412-7.
9. Park MH, Choi MS, Kwak DH, Oh K, Yoon DY, Han SB, *et al*. Anti-cancer effect of bee venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-κB. Prostate.
10. Bellik Y. Bee venom: Its potential use in alternative medicine. Anti-Infective Agents. 2015;13(1):3-16. DOI:10.2174/2211352513666150318234624.
11. Moreno M, Giralt E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin, and mastoparan. Toxins. 2015;7(4):1126-50. DOI:10.3390/toxins7041126.
12. Gajski G, Garaj-Vrhovac V. Melittin: A lytic peptide with anticancer properties. Environ Toxicol Pharmacol. 2013;36:697-705. Doi: 10.1016/j.etap.2013.06.009.
13. Chen J, Guan SM, Sun W, Fu H. Melittin, the major pain-producing substance of bee venom. Neurosci Bull. 2016;32:363-370. DOI: 10.1007/s12264-016-0024-y.
14. Rady IA, Siddiqui IA, Rady M, Mukhtar H. Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. Cancer Lett. 2017;402:16-31. DOI: 10.1016/j.canlet.2017.05.010.
15. Zarrinnahad H, Mahmoodzadeh A, Hamidi MP, Mahdavi M, Moradi A, Bagheri KP, Shahbazzadeh D. Apoptotic effect of melittin purified from Iranian honey bee venom on human cervical cancer HeLa cell line. Int J Pept Res Ther. 2018;24:563-570. DOI: 10.1007/s10989-017-9641-1.
16. Dudler T, Chen WQ, Wang S, Schneider T, Annand RR, Dempcy RO, *et al*. High-level expression in Escherichia coli and rapid purification of enzymatically active honey bee venom phospholipase A2. Biochim Biophys Acta (BBA) - Lipids Lipid Metab. 1992;1165:201-210. DOI: 10.1016/0005-2760(92)90188-7.
17. Hossen M, Gan SH, Khalil M. Melittin, a potential natural toxin of crude bee venom: Probable future arsenal in the treatment of diabetes mellitus. J Chem. 2017;2017:4035626. doi: 10.1155/2017/4035626.
18. De Graaf DC, Brochetto Braga MR, De Abreu RMM, Blank S, Bridts CH, De Clerck LS, *et al*. Standard methods for *Apis mellifera* venom research. J Apic Res. 2021;60(4):1-31. doi:10.1080/00218839.2020.1801073.
19. Kuzmenkov AI, Peigneur S, Nasburg JA, Mineev KS, Nikolaev MV, Pinheiro-Junior EL, *et al*. Apamin structure and pharmacology revisited. Front Pharmacol. 2022;13:977440. doi:10.3389/fphar.2022.977440.
20. Khan N, Niazi ZR, Akhtar A, Khan MM, Khan S, Baloch N, *et al*. Hyaluronidases: a therapeutic enzyme. Protein Pept Lett. 2018;25(7):663-676. DOI: 10.2174/0929866525666180629121823.
21. Ahmed-Farid OA, Taha M, Bakeer RM, Radwan OK, Hendawy HAM, Soliman AS, *et al*. Effects of bee venom and dopamine-loaded nanoparticles on reserpine-induced Parkinson's disease rat model. Sci Rep. 2021;11(1):21141. DOI:10.1038/s41598-021-00764-y.
22. Schumacher MJ, Schmidt JO, Egen NB, Dillon KA. Biochemical variability of venoms from individual European and Africanized honey-bees (*Apis mellifera*). J Allergy Clin Immunol. 1992;90:59-65.
23. Mumu NN, Sarker M, Islam MR, Akand S, Bashar MA.

Some morphological aspects of Asian honeybee (*Apis cerana*) and isolation of its melittin content. J Biodivers Conserv Bioresour Manag. 2017;3(2):77-84.
24. Somwongin S, Chantawannakul P, Chaiyana W. Antioxidant activity and irritation property of venoms from Apis species. Toxicon. 2018;145:32-9.
25. Surendra NS, Jayaram GN, Reddy MS. Antimicrobial activity of crude venom extracts in honeybees (*Apis cerana*, *Apis dorsata*, *Apis florea*) tested against selected pathogens. Afr J Microbiol Res. 2011;5(18):2765-72.
26. Małek A, Strzemski M, Kurzepa J, Kurzepa J. Can bee venom be used as anticancer agent in modern medicine? Cancers. 2023;15(16):3714. DOI:10.3390/cancers15163714.
27. Ceremuga M, Stela M, Janik E, Gorniak L, Synowiec E, Sliwinski T, *et al*. Melittin-A natural peptide from bee venom which induces apoptosis in human leukaemia cells. Biomolecules. 2020 Feb 6;10(2):247. DOI: 10.3390/biom10020247. PMID: 32041197; PMCID: PMC7072249.
28. Moon DO, Park SY, Choi YH, Kim ND, Lee C, Kim GY. Melittin induces Bcl-2 and caspase-3-dependent apoptosis through downregulation of Akt phosphorylation in human leukemic U937 cells. Toxicon. 2008 Jan;51(1):112-20. DOI: 10.1016/j.toxicon.2007.08.015. Epub 2007 Sep 2. PMID: 17936321.
29. Zhao J, Hu W, Zhang Z, Zhou Z, Duan J, Dong Z, *et al*. Bee venom protects against pancreatic cancer via inducing cell cycle arrest and apoptosis with suppression of cell migration. J Gastrointest Oncol. 2022;13(2):847-858.
30. Khamis AA, Ali EMM, Salim EI, El-Moneim MAA. Synergistic effects of bee venom, hesperidin, and piperine with tamoxifen on apoptotic and angiogenesis biomarker molecules in breast cancer. Cancer Cell Int. 2023;23(1):110. DOI:10.1186/s12935-023-02864-7.
31. Cai HY, Fu XX, Jiang H, Han S. Adjusting vascular permeability, leukocyte infiltration, and microglial cell activation to rescue dopaminergic neurons in rodent models of Parkinson's disease. NPJ Parkinson's Dis. 2021;7(1):91. doi:10.1038/s41531-021-00233-3.
32. Mi W, Meng M, Xu F, Sun L. Efficacy of acupuncture as adjunct therapy for sleep disorders in Parkinson's disease: a systematic review and meta-analysis. Complement Ther Med. 2024;82:103044. DOI:10.1016/j.ctim.2024.103044.
33. Ye M, Chung HS, Lee C, Yoon MS, Yu AR, Kim JS, *et al*. Neuroprotective effects of bee venom phospholipase A2 in the 3xTg AD mouse model of Alzheimer's disease. J Neuroinflammation. 2016;13:10-12. DOI:10.1186/s12974-016-0476-z.
34. Proulx É, Power SK, Oliver DK, Sargin D, McLaurin J, Lambe EK. Apamin improves prefrontal nicotinic impairment in a mouse model of Alzheimer's disease. Cereb Cortex. 2020;30(2):563-74. DOI:10.1093/cercor/bhz10.
35. Yang L, Zhao W, Gong X, Yue D, Liu Y, Tian Y, *et al*. Exploring potential network pharmacology- and molecular docking-based mechanism of melittin in treating rheumatoid arthritis. Medicine. 2023;102(32). doi:10.1097/MD.0000000000034728.
36. Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. J Clin Invest. 2015;125(6):2228-33. doi:10.1172/JCI78088.
37. Mohammadi-Rad M, Ghasemi N, Aliomrani M. Evaluation of apamin effects on myelination process in C57BL/6 mice model of multiple sclerosis. Res Pharm Sci. 2019;14:424.
38. Kim JY, An HJ, Kim WH, Park YY, Park KD, Park KK. Apamin suppresses biliary fibrosis and activation of hepatic stellate cells. Int J Mol Med. 2017;39(5):1188-94. doi:10.3892/ijmm.2017.2922.
39. Kim JY, Lee WR, Kim KH, An HJ, Chang YC, Han SM, *et al*. Effects of bee venom against Propionibacterium acnes-induced inflammation in human keratinocytes and monocytes. Int J Mol Med. 2015;35:1651-1656. doi:10.3892/ijmm.2015.2180.
40. Hossen M, Gan SH, Khalil M. Melittin, a potential natural toxin of crude bee venom: probable future arsenal in the treatment of diabetes mellitus. J Chem. 2017;2017:4035626. doi:10.1155/2017/4035626.
41. Ivanov E, Akhmetshina M, Erdiakov A, Gavrilova S. Sympathetic system in wound healing: multistage control in normal and diabetic skin. Int J Mol Sci. 2023;24(3):2045. doi:10.3390/ijms24032045.
42. Reuter S, Raspe J, Taube C. Microbes little helpers and suppliers for therapeutic asthma approaches. Respir Res. 2024;25(1):29.
43. Kim S, Min K, Yun S, Hwang D, Kang H. Therapeutic dose, duration, and efficacy of bee venom for treating clinical mastitis in dairy cows. J Vet Clin. 2023;41:8-17.
44. Jeong CH, Cheng WN, Bae H, Lee KW, Han SM, Petriello MC, Lee HG, Seo HG, Han SG. Bee venom decreases LPS-induced inflammatory responses in bovine mammary epithelial cells. J Microbiol Biotechnol. 2017;27(10):1827-36. DOI:10.4014/jmb.1706.06003.
45. Tsai LC, Lin YW, Hsieh CL. Effects of bee venom injections at acupoints on neurologic dysfunction induced by thoracolumbar intervertebral disc disorders in canines: a randomized, controlled prospective study. Biomed Res Int. 2015;2015:363801. doi:10.1155/2015/363801.
46. Vick JA, Mehlman B, Brooks R, Phillips SJ, Shipman W. Effect of bee venom and melittin on plasma cortisol in the unanesthetized monkey. Toxicon. 1972;10:581-6. doi:10.1016/0041-0101(72)90119-5.
47. Turn JT, Mayer J, Nagata K, Banovic F, Meichner K, Hurley DJ, *et al*. Impact of apitherapy on canine, equine, and chicken lymphocytes, *in vitro*. Vet Immunol Immunopathol. 2024;268:110700. DOI:10.1016/j.vetimm.2023.110700.
48. Bava R, Castagna F, Musella V, Lupia C, Palma E, Britti D. Therapeutic use of bee venom and potential applications in veterinary medicine. Vet Sci. 2023;10(2):119.
49. Benton AW, Mores RA, Stewart JB. Venom collection from honeybees. Science. 1963;142:228-30.
50. Omar RE. Effect of bee venom collection on the measurement of brood rearing activity of honey bee colony *Apis mellifera* L. Middle East J Agric Res.

2017;6:409-14.
51. Han SM, Lee KG, Yeo JH, Kweon HY, Woo SO, Lee ML, *et al*. Device for collecting bee venom. WIPO Patent WO2007037566A1; c2007 Apr 5.
52. A trap for the collection of honey bee venom. European Patent EP2514306B1; c2012 Oct 24. Available from: https://patentimages.storage.googleapis.com/38/d3/f3/cb9d7cf51dcb34/EP2514306A1.pdf (accessed 2019 Sep 9).
53. Han S. Device for collecting apitoxin. Korean Patent KR2014-097515-1; c2014 Jun 13.
54. Ahn C. Device for collecting apitoxin. Korean Patent KR10-2012-0048581; c2012 May 8.
55. Robson CH. Bee venom collection apparatus. U.S. Patent US4739531; c1988 Apr 26.
56. Serrinha V, Correia S, Marques G. Productivity and economic analysis of a new intensive collector in the Portuguese market with implication of open innovation perspective. J Open Innov Technol Mark Complex. 2019;5:1-18. doi:10.3390/joitmc5030071.
57. Abdulraouf MA, Mohamed DS. Impact of bee venom collector equipment positions on the productivity of venom extracted from Carniolan, Buckfast, and Italian honeybee hybrids in Upper Egypt. Egypt Acad J Biol Sci A Entomol. 2023;16(1):189-97. DOI:10.21608/eajbsa.2023.295885.
58. Muller UR. Insektenstichallergie. Klinik, Diagnostik und Therapie. Stuttgart: Gustav Fischer Verlag; c1988.
59. Shkenderov S, Ivanov T. Pcelni Produkti, The Bee Products (in Bulgarian). Sofia: Zemizdat; c1983.
60. de Graaf DC, Brochetto Braga MR, Magalhães de Abreu RM, Blank S, Bridts CH, De Clerck LS, *et al*. Standard methods for *Apis mellifera* venom research. J Apic Res. 2021;60(4):1-31. DOI:10.1080/00218839.2020.1801073.
61. Pucca MB, Cerni FA, Oliveira IS, Jenkins TP, Argemí L, Sørensen CV, *et al*. Bee updated: Current knowledge on bee venom and bee envenoming therapy. Front Immunol. 2019;10:2090. DOI:10.3389/fimmu.2019.02090.
62. Guralnick MW, Mulfinger LM, Benton AW. Collection and standardization of Hymenoptera venoms. Folia Allergol Immunol Clin. 1986;33:9-18.
63. Zareski R, Nackov K, Gjoshe S. Economic prospects in the production of bee venom and bee venom products from the aspect of application in medical and cosmetological therapies. J Biomed Res Stud. 2022;2(1):101.