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Bee Venom and its Biological Effects

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Abstract

Apitherapy is defined as "the use of *Apis mellifera* L. products such as royal jelly, pollen, honey, propolis, beeswax, and bee venom (BV) in the treatment of ailments". Although honey is the primary product acquired, other bee products are also obtained in Turkey. These commodities, in addition to being utilized as nutrition, have been employed to promote human health since ancient times owing to the biologically active compounds they contain. BV is increasingly commonly used in apitherapy and has a wide range of biological effects including antiviral, antidiabetic, anticancer, antirheumatic, anticoagulant, antibacterial, anti-cancer, anti-aging, neuroprotective, analgesic, antioxidant, hepatoprotective, and anti-asthmatic properties. According to the literature, BV has promising biological implications for human health, which constitutes the topic of this review.

Key Words: Bee venom, apitoxin, apitherapy, bee venom acupuncture, melittin.

1. Introduction

Apitherapy is a complementary therapy that utilizes products of *Apis mellifera* L., also known as the honey bee, such as honey, pollen, propolis, royal jelly, beeswax, and bee venom (BV) to prevent or treat some disorders. Since the time of Ancient Egypt and the Romans, bee-based products have been utilized to cure ailments, maintain well-being and prevent diseases (Cherbuliez, 2013). Although all of the aforementioned bee components are employed in apitherapy, BV is the product that seems most widely used today to treat various diseases. BV, also known as apitoxin, is a light-colored, odorless, acidic, and poisonous animal secretion with a pungent and bitter taste, which is stored in the venom sacs of female worker bees and aids in their defense against the predators. BV contains a wide range of active compounds such as enzymes, peptides, non-peptide components, and physiologically active amines, where it consists of 88% water and just 0.1 µg of dry venom. Dry venom made up complex peptides (particularly melittin, apamin, adolapin, mast cell degranulating peptide, minimine, secarpine, melittin F, cardiopep etc.), biologically active amines (histamine, epinephrine, dopamine, noradrenaline etc.), enzymes (phospholipase A2, hyaluronidase,

phosphomonoesterase, lysophospholipase, phospholipase B, α-D-glucosidase etc.), lipids, carbohydrates (glucose, fructose etc.), free amino acids (glycine etc.), minerals (potassium, calcium, magnesium), volatile substances and organic acids (citric acid, malic acid, malonic acid etc.). Changes in the chemical composition of BV can be seen as a result of bee species, location, and dietary changes. For this reason, it is critical to standardize BV and evaluate its toxicity before using it for medical purposes (Son et al., 2007; Sung et al., 2021; Wehbe et al., 2019). The composition of dry BV is detailed in Table 1.

BV therapy has been used in the treatment of various ailments such as rheumatism and arthritis since ancient times. There are numerous BV treatments available including bee sting therapy, BV acupuncture (BVA), directly injection of BV into human body and the use of BV products externally. Bee sting treatment is injecting BV into human skin *via* live bee stings. However, this treatment technique has a significant chance of causing undesirable side effects. BVA is a pharmacopuncture treatment derived from bee sting treatment that is effective in the treatment of diseases such as rheumatoid arthritis, chronic low back pain, acne, diabetes, Parkinson's disease, Alzheimer's disease, and asthma by injecting diluted BV into acupuncture points. Both the pharmacological effects of BV and the stimulation of acupuncture points elicit a response. On the other hand, BVA includes injecting BV diluted with saline into a specific acupoint, has been used to treat many forms of pain in the clinical domains of traditional, complementary, and alternative medicine and does not have the same hazards as bee sting therapy (Seo et al., 2017; Sung & Lee, 2021; Wehbe et al., 2019).

The idea of using BV in medicine originated, when beekeepers suffered from ailments such as rheumatism and joint pain (Wehbe et al., 2019). The effects of BV on a range of ailments were then studied using various treatment techniques. BV has been shown to have analgesic, anti-cancer, anti-asthmatic, antioxidant, anti-aging, anti-atherosclerotic, anti-diabetic, hepatoprotective, antivira, neuroprotective and anti-rheumatoid arthritis biological activities as a result of scientific research, and its use in the cosmetic industry has recently increased.

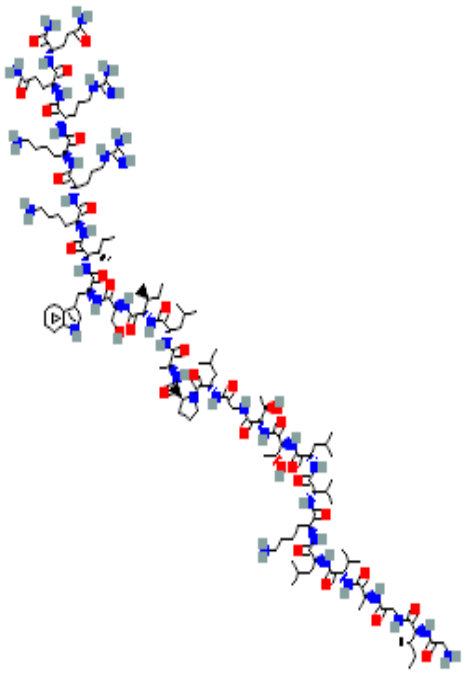
2. Method

A literature search was undertaken to evaluate the biological activity of BV by using various filters to search English papers in Scopus, Web of Science, Google Scholar Library, and PubMed. The following search phrases were used: bee venom, bee venom acupuncture, melittin, and bee venom treatment.

3. BV composition

BV has a very complex structure. The main components have been mentioned below.

3.1. Melittin



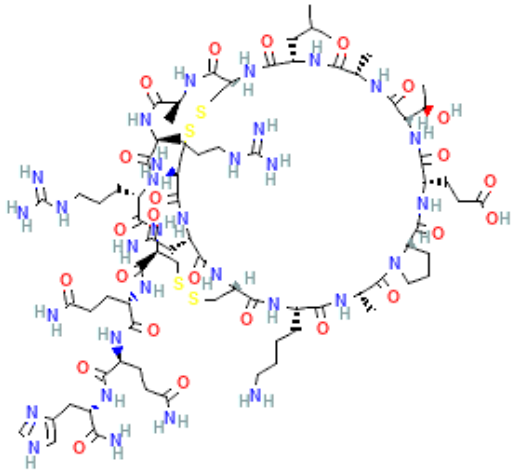
The 2D structure of melittin (PubChem CID: 16133648 was downloaded from the PubChem databank)

Table 1: BV components

Molecule type	Components	References
Polypeptides	Melittin (50%), apamin (2%), adolapin (1%), mast cell degranulating peptide (2-3%), minimine (2-3%), procamine A-B, secarpine, melittin F, cardiopep	(Azam et al., 2018; Bellik, 2015; Fenard et al., 1999; H. Y. Kim et al., 2020; Lee & Bae, 2016; Moreno & Giralt, 2015; Shimpi et al., 2016; Sığ et al., 2019; Son et al., 2007; Wehbe et al., 2019; Yong et al., 1990)
Biologically active amines	Histamine, epinephrine, dopamine, noradrenaline, serotonin	(Bellik, 2015; Han et al., 2000; Hossen et al., 2017; Nielsen, 2020; Rakha et al., 2018; Sığ et al., 2019; Son et al., 2007; Yasin et al., 2000; Yong et al., 1990)
Enzymes	Phospholipase A2 (12-15%), hyaluronidase (1-2%), phosphomonoesterase, lysophospholipase, α-D-glucosidase (0.6%), phospholipase B (1%)	(Bellik, 2015; Han et al., 2000; Hossen et al., 2017; Kemeny et al., 1984; Lee & Bae, 2016; Pattabhiramaiah et al., 2020; Shimpi et al., 2016; Sığ et al., 2019; Wehbe et al., 2019)
Carbohydrates	Glucose (2-4%), fructose	(Bellik, 2015; Han et al., 2000; Shimpi et al., 2016; Sığ et al., 2019; Wehbe et al., 2019; Ye et al., 2016)
Free amino acids	α-amino acids, β-aminoisobutyric acid	(Bellik, 2015; Han et al., 2000; Rady et al., 2017; Shimpi et al., 2016; Wehbe et al., 2019)
Minerals	Potassium, calcium, magnesium	(Bellik, 2015; Moreno & Giralt, 2015; Sığ et al., 2019; Wehbe et al., 2019)
Volatile substances	Complex ethers	(Bellik, 2015; Sarhan et al., 2020; Wehbe et al., 2019)
Organic acids (constitute 7-8% of the total chemical structure)	Citric acid, malic acid, succinic acid, fumaric acid, malonic acid, glutaric acid, and kynurenic acid	(Bellik, 2015; Han et al., 2000; Pawlak et al., 2020; Rady et al., 2017)

Melittin is a poisonous and allergic peptide with a molecular weight of 3850 Da and 26 amino acid residues that makes up half of the dry BV. It is in charge of cell lysis, determining the toxicity of BV, and inducing itching and irritation. The amino terminal section (residues 1-20) is hydrophobic and has no lytic activity, whereas the carboxy terminal section (residues 21-26) is hydrophilic and has lytic action. Melittin's amphoteric feature renders it soluble in water as a monomer. Melittin peptide accumulation causes cell lysis by altering the phospholipid structure in the cell membrane. Melittin possesses anti-cancer, anti-inflammatory, antiviral, antibacterial, and neuroprotective properties (Lee & Bae, 2016; Son et al., 2007; Yong et al., 1990). Some viruses are coated in a membrane that is similar to the host's cell membrane and includes specific viral proteins. Enveloped viruses easily evade the host's immune system and that makes their treatment more difficult. Melittin was shown to have an antiviral impact on enveloped viruses such as retroviruses and herpesviruses in a research by causing membrane lysis surrounding encapsulated viruses (Fenard et al., 1999; Yong et al., 1990). Several studies are available in the literature that examine anticancer activity of melittin. Anticancer activity is the most notable effect of melittin as well as BV, since several researches pointed out to its substantial anticancer activity in the majority of the investigations. According to the findings, melittin shows its anticancer activity through suppressing TLR2, TLR4, CD14, NEMO, and PDGFRβ signaling pathways and activating p38, ERK1/2, AKT, and PLCγ1 pathways, increasing calcium channel activation, activating death receptors (DR4, DR5), and indirectly stimulating caspase 3 and caspase 9 enzymes that play a role in apoptosis (Lee & Bae, 2016; Wehbe et al., 2019).

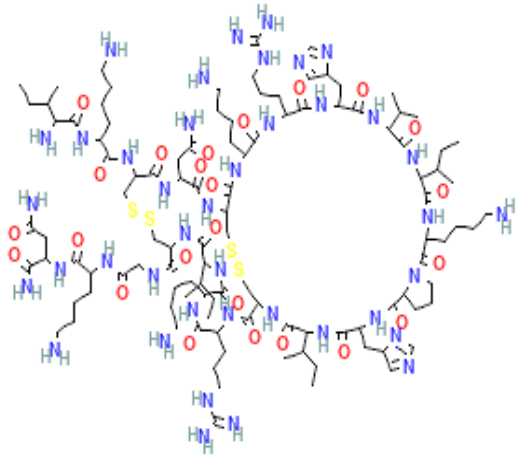
3.2. Apamin



The 2D structure of apamine (PubChem CID: 16133797 was downloaded from the PubChem databank)

Apamin is a blood-brain barrier-crossing polypeptide made up of 18 amino acids that accounts for less than 2% of the dry venom's weight and contains two disulfide bridges (Azam et al., 2018; H. Y. Kim et al., 2020). It is also an antioxidant and anti-inflammatory substance. In a study in which a lipopolysaccharide-induced acute kidney injury model was generated in male C57BL/6N mice, it was revealed that the kidney dysfunction and histological damage, particularly tubular damage, of the mice in the treatment group improved significantly compared to the control group after being injected with apamin at a dose of 10 mg/kg. This effect has been linked to a considerable decrease in tubular cell apoptosis caused by apamine-induced caspase-3 activation and TLR-4-mediated regulation of cytokine production. Apamin was also shown to reduce LPS-induced oxidative stress *via* altering the expression of nicotinamide adenine dinucleotide phosphate oxidase 4 and heme oxygenase-1 in the same research (J. Y. Kim et al., 2020).

3.3. MCD (Mast cell degranulating) peptide



The 2D structure of mast cell degranulating peptide (PubChem CID: 16132290 was downloaded from the PubChem databank)

It is the neurotoxic component of BV and accounts for 2-3% of dry BV. It has a molecular structure that is similar to apamine and comprises of 22 amino acid residues. MCD was proven to cause a remarkable decrease in blood pressure in rat experiment. In this context, it is the component considered to be responsible for the hypotension seen in BV intoxication (Wehbe et al., 2019).

3.4. Adolapin

It is a polypeptide of 103 amino acids that accounts for around 1% of dried BV. Adolapin has anti-inflammatory, anti-nociceptive, and antipyretic activities by reducing cyclooxygenase activity and blocking prostaglandin synthesis (Bellik, 2015; Cherniack & Govorushko, 2018).

3.5. Phospholipase A2

BV phospholipase A2 (BV-PLA2) is the alkaline component that accounts for 12-15% of dry BV, includes 128 amino acid residues, and 4 disulfide bridges, while it possesses the maximum lethality. During the

erythrocyte lysis process, it interacts synergistically with melittin, creating gaps in the cell membrane and allowing melittin to flow through, killing the cells by breaking down the phospholipid layers, which are the major components of the cell membrane. It has antiparasitic, anticancer, and anti-inflammatory properties (Dudler et al., 1992; Hossen et al., 2017; Wehbe et al., 2019).

3.6. Phospholipase B

It is detected in a 1% concentration in dried BV, which stimulates PLA2 activity (Hossen et al., 2017).

3.7. Hyaluronidase

It is a polypeptide with 350 amino acid residues that makes up 1-2% of BV and selectively breaks down hyaluronic acid in the extracellular matrix of the skin, allowing other venom components to enter the body. It is known that it stimulates blood vessel dilation, increasing permeability, and so boosting blood circulation, which therefore enhances BV circulation (Hossen et al., 2017).

4. Safety of BV for human

BV causes allergic reactions after application. These reactions might emerge in the skin, respiratory tract, cardiovascular system or gastrointestinal tract. Severe anaphylactic shock can then result in cerebral or myocardial ischemia. The proteic components in the venom are considered to trigger these allergic reactions. PLA2, melittin, and hyaluronidase are considered to be major allergens and inducers of immunoglobulin E (IgE) in BV. When BV venom is administered to a human body, symptoms such as dyspnea, limb paralysis, loss of consciousness, nausea, and exhaustion might occur. High amounts of BV (100 µg/mL) can induce human lymphocyte instability, although smaller concentrations are not genotoxic and do not cause oxidative damage. The skin sensitivity level of BV (10 mg/mL) was classified as Grade 1-poor in a skin sensitization study done on 50 guinea pigs using the Buehler test. As a result, it is advised that an allergy test and controlled application be performed prior to treatment with BV (W. R. Lee et al., 2011; Zhang et al., 1995). When BV is applied to the human body, mild side effects such as fatigue, localized edema, pruritus, skin rash nausea, and vomiting may occur, as can severe side effects such as limb paralysis, dyspnea, and loss of consciousness (Cheng & Ren, 2004; Ko et al., 2022; Zhang et al., 1995). According to a retrospective study conducted at the hospital between January 2010 and April 2019, only 0.175% of 8580 patients admitted to the hospital reported type 1 hypersensitivity and 0.047% anaphylactic shock (Lee et al., 2020). The incidence of anaphylactic shock seen during treatment with penicillin, an antibiotic that has been used for a long time in the treatment of many infections, has been reported to be between 0.02-0.04% (Patterson & Stankewicz, 2021). BVA therapy is currently limited due to the risk of anaphylactic shock and other allergic responses during BV treatment. However, as compared to penicillin therapy, the rate of anaphylactic shock reported with BVA treatment is substantially lower.

5. The process of obtaining BV

BV can be obtained in three different ways. First, a device known as a BV collector is placed at the hive's entrance and then collected by scraping after enough BV has accumulated. This instrument uses low voltage electricity to acquire and extract a greater amount of BV from the bee. Another technique is to set an appliance for the bee's sting. The poison left by the bee is collected on the hive machinery. The third way is to use tweezers to mechanically retrieve the sacs in which the honey bee stores its venom (De Graaf et al., 2021). The collected BVs are purified and powdered using the lyophilization procedure in a specific pre-sterile environment. The gathered BV is

treated in a pre-sterile special environment before being refined. Lyophilized BV is powdered and diluted with distilled water at a certain quantity. BV is put into appropriate packaging, packed, sanitized, and packaged for clinical usage as pharmacopuncture injections (Sung et al., 2021).

6. Biological effects of BV

6.1. Anti-acne activity

Acne vulgaris is an inflammatory dermatological disorder characterized by excessive sebum production, aberrant follicular keratinization, and proliferation of *Propionibacterium acnes*, which is an anaerobic and gram positive bacteria on skin microbiota. *P. acnes* causes an increase in the production of proinflammatory cytokines such as IL-1ß, IL-8, and TNF-α (Han et al., 2016).

In a clinical study conducted on 30 participants aged 12-33 years, 77% of which are being Caucasian with acne problems. The facial serum containing 0.7-0.9 g of purified BV was applied on entire clean face of all participants twice a day, morning and evening, for 6 weeks. The Modified Cook's Acne Grading Scale was used to assess the effects of BV serum on acne. Changes in visible acne lesion counts following therapy with pure BV (PBV™) serum were recorded at 3 and 6 weeks, as well as changes in acne lesion numbers by image analysis. Acne is classified into 5 types: open comedones, closed comedones, papules, pustules, and nodules. According to the findings of this study, while all forms of acne improved after 3 and 6 weeks of treatment, open comedones had the most benefit. The administration of pure BV serum for 6 weeks resulted in a 92% in open comedones compared to the first day. The absence of allergic reactions or irritation in the participants throughout the research gives credence to the application's safety. After 6 weeks of applying pure BV serum to the skin, the reduction in open comedones, closed comedones, and papules was 92 %, 72.2 % and 43.4%, respectively. Interestingly, although all of the pustules vanished after 3 weeks, they reappeared after 6 weeks (Han et al., 2016).

A research on 30 eight-week-old ICR mice examined the effect of purified BV on *P. acnes*-induced dermatological illness. Mice were divided into 6 groups. While the control group received no intervention, the mice in the second group had their left ears injected intradermally with $1.0x10^7$ CFU of *P. acnes* in 20 µL of phosphate buffer solution (PBS) and an equal volume of PBS alone in their right ears, followed by 0.05 g vaseline. Following injections of *P. acnes* into both the right and left ears of the mice in the other groups, vaseline containing 1 µg, 10 µg, and 100 µg pure BV was administered topically to only the right ear of each mouse. At the end of 24 hour-period, histological examinations were performed. Following *P. acnes* injection, swelling, erythema, and redness, were observed to develope in mice ears resulting in significant production of pro-inflammatory cytokines such as TNF-α and IL-1ß. Although BV treatment decreased edema, erythema, and redness induced by *P. acnes*, the group treated with 1 µg BV exerted the greatest improvement. As a result of this observation, it can be stated that the side effects of using high concentrations of BV appear. The administration of BV effectively inhibited the secretion of pro-inflammatory cytokinins such as TNF-α and IL-1ß, according to western blot analysis (An et al., 2014).

Previous research has revealed that the increase in pro-inflammatory cells following *P. acnes* treatment was mediated by TLR2 (Kim et al., 2002). The double immunofluorescence investigation found that BV suppressed TLR2 expression and reduced the release of proinflammatory cytokines. Similarly, BV has been demonstrated to suppress the activation of transcription factors involved in the secretion and regulation of pro-inflammatory

cytokines such as AP-1 and NF-κB (An et al., 2014).

In vitro and *in vivo* experiments were conducted to explore the effect of mellitin, the primary component of BV, on *P. acnes*-induced inflammatory skin disease. In a research on *P. acnes*-induced HaCaT cells, 1 µg/mL mellitin dramatically reduced the levels of pro-inflammatory cytokines TNF-α, IL-1ß, IL-8, and *IFN*-γ compared to the control group. It has been observed that mellitin regulates TLR activation on the same cell line and that mellitin reduces the p38 MAPK signal, which in turn reduces the production of pro-inflammatory cytokines. The same group of researchers investigated the effect of mellitin on *acne vulgaris* in an animal model. Mellitin was demonstrated to suppress the production of TNF-α and IL-1ß cytokines in 8-week-old ICR mice infected with *P. acnes* through regulating the AP-1 and NF-κB transcription factors (Lee et al., 2014). As a result of all these studies, it sheds light on the fact that BV applied in the right dose can be effective in skin infection caused by *P. acnes*, in which the effective substance may be mellitin. Oral and topical antibiotic therapy is used for a long time in the treatment of *acne vulgaris*. This treatment method not only causes the development of antibiotic resistance, but also causes side effects such as diarrhea, nausea, and skin rashes. *In vitro* studies have been supported by *in vivo* studies and the effect of BV on the treatment of *acne vulgaris* has also been demonstrated by clinical studies. These developments can be an alternative to the use of corticosteroids, retionoids, and antibiotics in the treatment of *acne vulgaris* and can be a new perspective for both pharmaceutical and the cosmetic industries. The pathways through which anti-acne effect of BV takes place should be supported by more detailed studies.

6.2. Antiviral activity

Although infectious illnesses have always played an important role in human history, the new coronavirus SARS-CoV-2 virus that emerged in the Wuhan, Hubei Province in December, 2019 affected the entire world. Although treating viral infections such as COVID-19, hepatitis, AIDS, and influenza is challenging, there is sometimes no particular medicine to cure. The contagiousness of viral infections is also extremely dangerous to public health. The virus infects the host replicates its genetic material in the host cell. As a result, in the treatment of viral illnesses, a selective impact is essential; otherwise, antiviral medications may potentially possesses a mutagenic effect on the host cell. Today, the chronic and acute toxicity of antiviral medications used in the clinic, their inadequacy in therapy, the rapid evolution of virus resistance to these treatments, and the high cost of the drugs all contribute to the hunt for novel antiviral compounds (Chan et al., 2020; Musarra Pizzo et al., 2021).

According to the results of a survey of 5115 beekeepers, 723 of whom were in Wuhan, the epicenter of the COVID-19 outbreak, by the local beekeepers association in China, the beekeepers did not show any symptoms of COVID-19 and their general health was quite good. It was determined that 3 out of 121 patients treated with apitherapy between October 2019 and December 2019 were not infected with SARS-CoV-2 despite being in close contact with patients infected with SARS-CoV-2 (Yang et al., 2020). The researchers, who conducted the mentioned study, believed that the common thread across all of these patients was that they developed a tolerance to BV, which triggers a severe allergic reaction. They emphasized that this research should be carried out on monkeys. It was hypothesized that the regulatory T cells were differentiated because immunization against BV developed in people exposed to BV or because it works as an ACE receptor blocker or as an ACE2 inhibitor against the ACE receptors utilized by the novel coronavirus (SARS-CoV-2) to enter cells. Melittin, a component of BV, exhibits antiviral properties by puncturing the protective membrane envelopes

surrounding viruses such as SARS-CoV-2 (Kasozi et al., 2020; Yang et al., 2020). In contrast to this study, a study conducted in Germany with 234 participants who are members of the German Beekeepers Association found that 2 beekeepers died as a result of COVID-19, while 42 beekeepers were infected with the SARS-CoV-2. Furthermore, the same study discovered that beekeepers' age, the number of bee stings in 2020, the total number of bee stings so far, gender, and whether or not they had any comorbidities had no effect on their immunity. The hypothesis which was conducted in China that beekeepers would be somehow immune to the illnesses caused by SARS-CoV-2 was disproved as a consequence of this investigation (Männle et al., 2020).

Antiviral effect of BV on the blood-borne hepatitis C (HCV) virus, which causes liver damage, was examined in the human hepatoma-derived Huh7it-1 cell line in a study. BV at various dilutions (0.01, 0.1, 1, 10, 100, and 1000 ng/mL) was found to have dose-dependent anti-HCV efficacy in this cell line. The IC_{50} value of BV was determined to be 0.05 ng/mL, and it was discovered to have very significant anti-HCV activity.
To ascertain which component for antiviral activity was responsible, efficacy of BV to replicate hepatitis C virus was considerably low following proteinase K protein removal. Phospholipase A2 is one of the enzymes found in BV (Sarhan et al., 2020). Previously, it was considered that the compound responsible for anti-HCV activity investigation of snake venom was due to phospholipase A2, which destroys the virus' envelope and coated with the envelope having a phospholipid composition (Chen et al., 2017). To evaluate if BV's anti-HCV action includes enzymatic activity, BV (100 ng/mL) was heated at 95 °C for 30 minutes, and at the conclusion of the treatment, it was figured out that the active compound was a material resistant to 95 °C, with no impact attributable to enzymatic activity. It is hypothesized that the molecule responsible for the action has a proteic structure, whereas it has a very low molecular weight. Melittin, apamin, and mast cell-degranulating peptide, which are among the primary peptides in BV, were treated and incubated separately with Hepatitis C virus to evaluate the substance in the content of the anti-HCV effectiveness of BV. It has been discovered that melittin, apamin, and the mast cell-degranulating peptide do not directly contribute anti-HCV efficacy in BV (Sarhan et al., 2020). When the impact of BV on the bovine viral diarrhea virus was previously explored, it was shown that mellitin did not have a direct anti-viral effect, but rather enhanced the anti-viral action, when combined with apamin (Picoli et al., 2018).

Melittin is responsible for the antiviral activity of BV according to research on *herpes simplex* virus-1 and -2 (Yasin et al., 2000). As a result of these investigations, it has been stated that the molecule responsible for anti-HCV action may not be melittin, apamin or mast cell-degranulating peptide. BV may have a direct antiviral activity (Sarhan et al., 2020). Melittin acts as an antiviral agent by destroying the protective membrane envelopes that enclose viruses such as human immunodeficiency virus (HIV) (Kasozi et al., 2020).

6.3. Analgesic activity

BV acupuncture has been studied for its benefits on musculoskeletal pain such as shoulder pain and low back pain, inflammatory pain such as arthritis pain, neuropathic pain such as post-stroke pain, prostate discomfort, and chronic regional pain (Sung & Lee, 2021). Chronic low back disease or pain is a common and frequently recurring condition among people without a pathological cause, limiting movement and worsening quality of life (Andersson, 1999). In a randomized, double-blind, and placebo-controlled clinical trial, 54 volunteer patients were divided into 2 groups. While one group received just nonsteroidal anti-inflammatory

drug (NSAID), the other group received increasing quantities (0.2 mL, 0.4 mL, 0.8 mL) of BV + NSAID to the acupuncture sites once in each week. At the end of 12 week-period, when the participants' pain level was measured using the visual analogue scale, it was stated that the data of the group treated with BV acupuncture were superior. Other outcomes examined by VAS, Oswestry Disability Index (ODI), EuroQol 5 Dimension (EQ-5D), and Beck's Depression Inventory (BDI) included pain intensity, movement restriction, quality of life, and depression. It was discovered that after 3 weeks of BV acupuncture therapy, pain severity and back pain dysfunction were dramatically reduced. As a result, BV acupuncture therapy has been found to be beneficial in pain relief (Seo et al., 2017). Several investigations have shown that BV acupuncture has analgesic and antinociceptive effects that are mediated *via* α_2-adrenergic and serotonergic receptors (Kim et al., 2005; Kwon et al., 2001).

In a study, 80 Sprague–Dawley rats were subjected to collagen-induced arthritis. The tail wagging action of the experimental animals was significantly decreased after the development of rheumatoid arthritis. When compared to the non-treatment group, there was a considerable increase in tail wagging in the control group which was treated by applying simply saline to the identical acupuncture locations treated with BV acupuncture. The highest analgesic impact of BV acupuncture was observed 30 minutes after injection, and this effect was reported to persist at least 60 minutes. Because no analgesic effect was detected in the control saline-treated group, antinociceptive effect of BV acupuncture was determined to be produced by BV rather by acupuncture. In the same study, yohimbine as an indole alkaloid and α_2-adrenergic receptor antagonist along with *mu*-opioid receptor antagonist naloxone were administered to experimental animals prior to BV acupuncture administration to determine the mechanism of action of BV acupuncture treatment's analgesic effect on collagen-induced inflammatory pain. Finally, analgesic effect of BV acupuncture therapy was entirely negated by intraperitoneal administration of yohimbine. Intraperitoneal injection of the mu-opioid receptor antagonist; naloxone had no effect on analgesic efficacy on BV acupuncture. These findings imply that BVA's antinociceptive impact on inflammatory pain is specifically mediated *via* the α_2-adrenergic route rather than the opioidergic system (Baek et al., 2006). BVA owns a strong analgesic effects *via* activating spinal α_2-adrenoceptors (Kang et al., 2011). In the dorsal area of male Sprague-Dawley rats, a formalin-induced acute pain model was generated. While FOS gene expression increased in all regions of the lumbar spinal cord following formalin administration, it was inhibited early in the group treated with high-dose (0.08 mg/kg) BVA. According to this study, analgesic activity of BV may possibly be attributable to inhibition of c-FOS gene expression (Kim et al., 2003).

BVA administration (61.1 F 9.1 pg/mL) has been demonstrated in a rat model of collagen-induced rheumatoid arthritis to dramatically suppress IL-6 rise, promote apoptosis *via* a drop in BCL2 expression, and an increase in BAX and CASP3 expression in the rheumatoid synovium (Hong et al., 2005). Similarly, in a study of rheumatoid arthritis was induced in male Wistar rats with Freund's complete adjuvant (FCA) and type-2 collagen. Each experimental animal received BVA administration at a dosage of 1 mg/200 g and treatment with BV was demonstrated to reduce proinflammatory markers such as TNF-α and IL-6 (Nipate et al., 2015).

6.4. Anti-aging activity

The skin is the human body's biggest organ. Skin aging is a natural process defined by skin dehydration and wrinkles caused by genetic and cellular aging, collagen alteration as an intrinsic factor as well as extrinsic

factors such as prolonged sun exposure, UV exposure, nutrition, smoking, stress, lifestyle, using the wrong skin product, and so on. The decrease in collagen production and rise in its decomposition leads to a loss in skin flexibility and the collapse of fibroblasts, resulting in the formation of wrinkles (Han et al., 2015; Mesa-Arango et al., 2017). Facial serums containing BV at a concentration of 0.006 % were administered twice daily to the full face as 4 mL *per* application to 22 South Korean female volunteers aged 30 to 49 years with mild to moderate indications of photoaging. The overall wrinkle area on the skin, total number of wrinkles, and average wrinkle depth all decreased after 12 weeks administration of the serum. After 12 weeks, 54.6 % of the volunteers reported a significant improvement in wrinkles on their faces. A face serum containing BV has been claimed to have clinical anti-aging benefits, although the mechanism of action has not been thoroughly elucidated (Lee & Bae, 2016). On the other hand, the use of BV in the cosmetic industry has increased in recent years. Nonetheless, it is discouraged since it has a marked potential for producing allergic responses. A research group reported that the phospholipase A2 is the enzyme component of BV that produces adverse effects such as allergic reactions and erythema on the skin. Furthermore, the cytotoxicity of both BV and phospholipase A2-free BV on UV-irradiated human keratinocyte (HaCaT) and human dermal fibroblast (HDF) cell lines were investigated. While cell lines exposed to 3 µg/mL standard BV for 2 hours, it showed a severe level of cytotoxicity. Otherwise, cell lines treated with phospholipase A2-free BV showed no cytotoxicity. Depending on the concentration, both BVs boosted the formation of type-1 procollagen. Induced MMP-1 and MMP-13 secretion in cell lines treated to UV-B irradiation was reduced by concentrations of 1.5 µg/mL and 3 µg/mL of regular BV and phospholipase A2-free BV, respectively. As a result, collagen degradation was avoided. The activation of ERK1/2 and p38 signaling pathways revealed the cell signaling mechanisms behind the anti-wrinkle effects of regular and phospholipase A2-free BV. *In vitro* experiments have demonstrated that phospholipase A2-free BV is the preferable one with less cytotoxicity and better efficiency on UVB-irradiated skin cells than the original one (Lee et al., 2015).

6.5. Wound-healing activity

The wound healing process is formed into the stages as follows: hemostasis, inflammation, proliferation or re-epithelialization, neovascularization-angiogenesis, and remodeling (Guo & DiPietro, 2010; Kurek Górecka et al., 2020). *Diabetes mellitus* (DM) was induced on 45 BALB/c mice by giving streptozotocin (STZ) for 5 days. The wound-healing efficacy of BV was investigated in mice with DM-induced lesions. BV accelerated wound repair process in diabetic mice by activating collagen expression, reduced oxidative stress in diabetic wounds by activating antioxidant defense mechanisms such as GSH Px, MnSOD, catalase, and regulated angiogenesis by activating ß-defensin-2 expression. When compared to animals in the control group, it was revealed that it accelerated wound closure (Hozzein et al., 2018).

In diabetic mice, BV enhances angiogenesis of new tissues formed during the wound healing process and upregulates TGF-α and VEGF expression. In the same study, BV reduced the release of proinflammatory cytokines such as IL-1, IL-6, and TNF-α in damaged tissues and also accelerated wound healing by decreasing the expression of ATF-3 and iNOS. BV also reduced the formation of reactive oxygen species. BV-induced wound healing has been shown to reduce caspase-3, -8, and -9 activity, which triggers apoptosis during the neovascularization and angiogenesis phases of wound healing process (Badr et al., 2016; Kurek Górecka et al., 2020). According to the findings of an *in vitro* study on the human epidermal

keratinocytes (HEK) cell line, BV at low doses (1 µg/mL) stimulates the proliferation and migration of keratocyte cells, while also lowering the level of released proinflammatory cytokines such as IL-8 and TNF-α (Han et al., 2013; Kurek Górecka et al., 2020).

6.6. Anti-hyperglycemic activity

DM is a life-threatening metabolic condition defined by chronic hyperglycemia caused by abnormalities in insulin production, insulin action on its receptors or both (Kharroubi & Darwish, 2015). Hyperglycemia induces enhanced glycation between sugars and proteins' free amino groups resulting in structural and functional alterations in proteins (Sen et al., 2005). Different concentrations of BV (10, 20, and 40 µg/mL) were administered to each sample for *in vitro* investigation to determine the effect of BV on hemoglobin glycation by incubating the hemoglobin in the presence of glucose. The amount of heme was evaluated by cleaving free amino groups with florescamine. The study concluded that BV reduces glycation-induced heme breakdown in hemoglobin. Because it has a considerable antiglycation impact. It has been also proposed to be developed as a natural therapy for glycation-related problems in DM (Behroozi et al., 2014).

6.7. Effect on lupus nephritis

Lupus nephritis is a frequent and potentially fatal consequence of systemic lupus erythematosus (SLE). ZB/W F1 female mice with idiopathic glomerulonephritis, proteinuria, and renal failure were treated once a week with a subcutaneous injection of 3 mg/kg of BV diluted in saline at a concentration of 0.5 mg/mL. In the control group of 10 mice, an equal amount of saline was injected. This practice lasted 12 weeks. The kidneys and spleens of surviving mice were isolated and evaluated at the end of the research. The renal functions worsened and severe proteinuria occurred in the control group but the mean urine protein level reduced in the BV-treated group. The majority of glomeruli in the saline-treated group showed class IV and V morphology with lupus membranous nephropathy glomerulus with spherical endocapillary and mesangial hypercellularity, early crescent formation, and subepithelial spine formation with the onset of mesangial and capillary sclerosis, when the kidneys of mice were examined by light microscopy at the end. However, in the BV-treated group several glomeruli were found to have somewhat aberrant histology. Serum levels of IG2a, IgG, and IgG3 as well as levels of proinflammatory cytokines such as TNF-α and IL6 were found to be lower. When BV-injected animals were compared to saline-injected mice, the CD4+ and CD25+ regulatory T cell population was dramatically enhanced. FOXP3 expression in CD4+ T cells, which suppressed the immunological response, has also been found to be increased in mice injected with BV (H. Lee et al., 2011).

6.8. Anti-asthmatic activity

Asthma is a potentially fatal inflammatory lung disease characterized by high number of CD4+ T cells. In a study, in which an allergic asthma model was induced in Balb/c mice by ovalbumin, injection of BV (0.1 and 1 microg/mL) increased the number of regulatory T cells in mice, suppressed the production of cytokines such as IL2, IL4 and IL13, and reduced peribronchial and perivascular inflammatory cell infiltrates. It has been shown that the expression of CD4 + CD25 + and FOXP3 from natural regulatory cells rose, whereas IgE levels in experimental animals' blood decreased significantly (Choi et al., 2013; Son et al., 2007).

6.9. Effect on amyotrophic lateral sclerosis

Amyotrophil is a lateral sclerosis illness characterized by aberrant accumulation of

mutant SOD1 protein aggregates. TLR4, CD14, and TNF-α were utilized in a research, in which BV was administered into the acupuncture points of human-SOD1 G93A transgenic mice with ALS (male hSOD1G93A transgenic mice) at dose of 0.1 μg/g 3 times a week during 2 weeks. A drastical decrease was shown in neuroinflammation as well as a suppress in neuroinflammation in mouse spinal cords (Cai et al., 2015). Bees were administered at dose of 0.1 μg/g each time in a study conducted in hemizygous transgenic B6SJL mice carrying a glycine-alanine base pair mutation at codon 93 of cytosolic Cu/Zn superoxide dismutase gene (hSOD1G93A) an animal model of amyotrophic lateral sclerosis. By suppressing microglia activation and phospho-p38 MAPK production in the central nervous system, venom injection improved motor function (Yang et al., 2010).

6.10. Effect on alopecia

Alopecia is a serious condition that affects people of all ages. The impact of BV on alopecia was studied in a work by administering different concentrations (0.001 %, 0.005 %, 0.01 %) and minoxidil 2%, as a positive control, to the dorsal skin of female C57BL/6 mice for 19 days. Effect of BV on balding was studied using mouse skins and human dermal papilla cells utilizing quantitative real-time PCR and Western blot analysis. BV stimulated hair growth and slowed the transition from anagen to catagen. When compared to controls, hair growth was dose-dependently enhanced in both anagen phase mice and dexamethasone-induced catagen phase mice. BV decreased the production of SRD5A2 gene, which encodes type II 5-reductase, a key enzyme in the conversion of testosterone to dihydrotestosterone. Furthermore, BV has been demonstrated to increase human dermal papilla cells (hDPCs) and several growth factors such as insulin-like growth factor-1 receptor, vascular endothelial growth factor, fibroblast growth factor-2 and 7 in hDPCs treated with BV. Proliferation was boosted in a dose-dependent manner, when compared to control group. Finally, BV has the potential to be a powerful 5-reductase inhibitor as well as hair growth stimulator. Low doses should be used just as bee and hair stroke with its dose is on the proper track because successful venom can generate an effective side at big doses and start the entire reaction. The bee poisonings utilized in this property had no negative effects (Park et al., 2016).

6.11. Anticancer activity

Melittin, which is one of most important components of BV, boosted cell proliferation in two human leukemia cell lines, *e.g.* acute lymphoblastic leukemia (CCRF -CEM and CCL -119, ATCC TM) and chronic myelogenous leukemia K-562 (CCL -243, ATCC TM). It has been shown to have anti-leukemic properties *via* blocking and promoting cell death. Simultaneously, melittin reduced calcium pump action by blocking calmodulin. This led to a raise in Ca^{2+} concentration, which is harmful to cells and finally leads to cell death (Chu et al., 2007; Lyu et al., 2019; Nielsen, 2020).

Several researches have revealed that melittin displayed antileukemic potential by activating caspase-3 and -7 resulting in the activation of apoptosis in both cell types. It caused cell death by apoptosis. Melittin has been also shown to trigger apoptosis in the cervix HeLa cell line. It decreased the growth of HeLa cell line in a dose- and time-dependent manner according to the results of MTT experiment used to determine *in vitro* cytotoxicity. Melittin has been shown in a research to possess cytotoxic effects towards human breast cancer cell line, human hepatocellular carcinoma (Li et al., 2006), prostate cancer (Park et al., 2011), and ovarian cancer cell lines (Jo et al., 2012).

6.12. Antimicrobial activity

Melittin and PLA2, both components of BV, provide antibacterial activity by stimulating hole creation on the infecting microorganism's surface. As the holes on the microbe enlarge, the cell's integrity is compromised its genetic material is destroyed, and divisional proliferation is halted (Wehbe et al., 2019). Minimum inhibitory concentration (MIC) of BV on 16 different *Salmonella* strains isolated from poultry (*e.g. S. newport*, *S. isangi*, *S. enterica* subsp. *salama*, *S. bardo*, *S. montevideo*, *S. infantis, S. stanleyville*, *S. ndolo*, *S. dabou*, *S. typhimurium*, and *S. enteritidis*) was determined using broth microdilution method. Each cell line was treated with BV diluted with water at a concentration of 4.096 µg/mL. Although the MIC value was generally 512 µg/mL, where *S. enterica* subsp*. salama* had the highest MIC value of 1024 µg/mL and one of the *S. typhimurium* lines had the lowest MIC value as 256 µg/mL. Simultaneously, apitoxin inhibited biofilm development in *Salmonella* and substantially eliminated preexisting biofilms. Biofilm production was decreased by BV from 68.22% to 27.66%. As a result, researchers have underlined that combining apitoxin with other antimicrobial medicines can lead to the creation of novel and effective treatments (Arteaga et al., 2019).

In a relevant study, disc diffusion assay technique was used to test antibacterial activity of the venom obtained from *A. mellifera*, which was dried through lyophilization and subsequently purified, at 25, 50, 75, and 100 µg/mL concentrations. In this experiment, gentamicin (10 µg/disc) was utilized as a positive control. Antimicrobial activity of BV against *Escherichia coli* and *Staphylococcus aureus* strains was dose-dependent, but not observed against *Pseudomonas aeruginosa* and *Bacillus subtilis.* At a concentration of 100 µg/mL, BV had even a greater antibacterial activity than that of gentamicin, which was employed as a positive control against *E. coli* (29.06 ± 1.31 mm) and *S. aureus* (17.51 ± 1.07 mm). In the same study, the LD_{50} value of BV was estimated to be 177.8 µg/mouse as a result of the investigation. As a result, at non-toxic and safe quantities, BV has a very good antimicrobial impact (Babaie et al., 2020).

7. BV dosage forms and components

BV has recently been employed in cosmetic products such as face serum, moisturizer, and face mask, owing to its anti-aging and anti-acne properties. Lyophilized BV powders are stored in sterilized glass vials and dissolved with physiological saline at health centers that provide BV treatment. Melittin possesses anticancer action, as previously stated. It is extremely toxic and hemolyzes red blood cells when administered into the human body at anticancer doses. Melittin was loaded into a dual secured nano-sting (DSNS) produced by the combination of a zwitterionic glycol chitosan and disulfide bonds in a research. In a research of multiple cancer cell lines, melittin-loaded DSNS at 5 mM concentration may kill nearly 100 percent of cancer cells while having no hemolytic impact (Cheng et al., 2015). The toxicity, non-specificity, and fast degradation of cytotoxic peptides such melittin make *in vivo* administration challenging. Perfluorocarbon nanoparticles loaded with melittin demonstrated an anticancer impact in a research by directly targeting cancer cells and inducing death in these cells (Soman et al., 2009).

8. Pharmacokinetic properties of BV and its components

Tc-labeled melittin was administered intravenously to C57BL/6 experimental mice both freely and in a nanoparticle carrier in a study to determine the pharmacokinetic properties of melittin, the most important component of BV. Blood samples were collected from experimental animals at various time intervals. The plasma half-lives of free melittin and nanoparticled melittin were determined to be 0.79 ± 0.05 minutes

and 4.22 ± 1.48 minutes, respectively. Their distribution volumes were determined to be 9.32 ± 1.54 ml and 2.94 ± 0.31 ml, respectively. By examining the tissue samples, it was discovered that free melittin immediately settled on the cell membrane and induced hemolysis, however, melittin loaded on nanoparticle did not. Melittin-loaded nanoparticles suppressed tumor development more efficiently than free melittin. Half-life and effect duration were prolonged by adding Melittin into the nanoparticle system. Because it may be particularly targeted to tumor cells, its action on tumor cells is enhanced and its toxicity is lowered indirectly (Soman et al., 2009). Another research evaluated the pharmacokinetic differences between intravenous and oral administration of BV in powder form. According to the statistics, impact of BV administered orally is quite modest since it passes *via* the gastrointestinal tract. The loss of impact might be related to enzymatic reactions and degradation in the digestive system's acidic environment (Xing et al., 2003).

9. Stability of BV

A study found that diluting BV 3000 and 4000 times can keep it stable for 12 months at ambient temperature and refrigerator temperature. However, in the same investigation, diluted BV maintained at ambient temperature for 12 months did not demonstrate antibacterial action against *Staphylococcus aureus*, although diluted BV stored in a refrigerator did. Impact of the component responsible for the antibacterial activity of BV reduces at room temperature, according to this study (Kang et al., 2003). Another study, conducted in 2021, assessed stability of melittin, a component of BV known to be responsible for several biological effects such as anticancer, antiviral, and anti-inflammatory. Melittin concentration of identical BV held at ambient temperature and in the refrigerator for 6 months was quantified using a reversed phase high performance liquid chromatography (HPLC) technique with a photo-diode array (PDA) detector in this study, and no significant difference was found. Melittin's remarkable stability, even at room temperature, lowers the storage costs for BV makers (Flanjak et al., 2021).

10. Economic importance of BV

A gram of BV costs between $30 and $300 depending on its purity, according to 2009 research. In recent years, BV has grown in appeal in both the pharmaceutical and cosmetic industries. In Türkiye, there is currently no regulation governing the manufacture of BV. As a result, the number of BV producers is extremely low. Although the use of bee products in cosmetics and health care has only just begun in Turkish, European, and Well Eastern nations are far advanced in this area. Because BV is not widely used or used in Türkiye, the number of BV manufacturers is limited. This market has not emerged for the manufacturers that are already manufacturing. According to a research conducted in Portugal, the profitability rate of facilities that continue production using traditional methods utilized in the manufacturing of BV is quite low, and it is extremely expensive for those who want to enter this field. As a result, the profit generated from BV production may be raised by adhering to existing BV production methods, improving the yield of the product acquired, and lowering the cost (Serrinha et al., 2019).

Conclusion

The use of bee products, particularly BV, in curing diseases and protecting people's well-being has been used since ancient Egypt and Rome. The growing awareness of nature in the world has made bee products, especially BV, increasingly important for curing diseases and protecting the well-being of people. BV therapy is very popular,

particularly in Far East countries such as South Korea and China; it is also practiced in several European countries nowadays. In Türkiye, BV therapy has been becoming more popular. As a result, the number of apitherapy clinics in Türkiye has increased in recent years. It is important to conduct a BV allergy test before beginning any type of treatment involving BV and to ensure that BV is standardized. The majority of BV is composed of melittin, a polypeptide with a polypeptide structure, followed by the PLA2. The anti-inflammatory action of BV has attracted the most attention from researchers, and several scientific studies have been conducted on the issue. Melittin was considered to be responsible for these biological activities in research on the biological activities of BV, and many investigations on melittin have been conducted. According to the findings, melittin exhibits anticancer, antiviral, anti-inflammatory, neuroprotective, and antibacterial properties. BV is now being used not just for medicinal purposes, but also for cosmetic purposes. BV is being employed in cosmetic preparations to benefit from its anti-aging, skin-regenerating, and moisture-retaining properties. More research is needed to completely understand the biochemical pathways of BV.

Abbreviations

ACE: Angiotensin-converting enzyme, **AKT:** Alpha-serine/Threonine Kinase, **ALS**: Amyotrophic lateral sclerosis**, AP-1:** Activator protein 1, **ATF3:** Activating Transcription Factor 3, **BAX**: BCL2-Associated X Protein, **BCL2:** B-cell lymphoma 2, **BDI**: Beck's Depression Inventory, **BV:** Bee venom, **BVA:** Bee venom acupuncture, **BV-PLA2:** Bee venom phospholipase A2, **CASP3:** Caspase-3**, CD-4**: Cluster of differentiation 4, **CFU:** Colony-forming units, **COVID19**: Coronavirus disease 2019 **Da**: Dalton, **DR 4/5**: Death receptors 4/5 **ERK1/2**: Extracellular signal-regulated kinase ½, **EQ-5D**: EuroQol 5 Dimension, **FCA**: Freund's complete adjuvant, **FOS:** *Fos* Proto-Oncogene **GSH Px:** Glutathione peroxidase, ***HaCaT:*** Cultured Human Keratinocyte cells, **HCV**: Hepatitis C virus, **HDF**: Human dermal fibroblast cell line, **HEK:** Human epidermal keratinocytes cell line, **iNOS:** Inducible nitric oxide synthase, **ICR:** Institute of Cancer Research, **IC_{50}:** The half maximal inhibitory concentration, **IgE:** Immunoglobulin E, ***IFN-γ***: Interferon gamma, **IL-1ß:** Interleukin-1 Beta, **IL-2**: Interleukin -2, **IL-4:** Interleukin -4, **IL-6**: Interleukin -6, **IL-8**: Interleukin -8, **IL-13**: Interleukin -13, **LD_{50}:** Lethal Dose, 50%, **MAPK**: Mitogen-activated protein kinase, **MCD peptide:** Mast cell degranulating peptide, **MIC**: Minimum inhibitory concentration, **MMP-1**: Matrix metalloproteinase-1, **MMP-13**: Matrix metalloproteinase-13, **MnSOD:** Manganese-dependent superoxide dismutase, **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, **NEMO**: Nuclear factor kappa-B essential modulator **NF-κB :** Nuclear factor kappa B, **NSAID:** Nonsteroidal anti-inflammatory medicine, **ODI:** Oswestry Disability Index, **PBS:** Phosphate buffer solution, **PBV™ :** Purified bee venom**, PCR:** Polymerase chain reaction, **PDGFRβ**: Platelet-derived growth factor receptor beta, **PLA2:** Phospholipase A2, **PLCγ1**: Phospholipase Cγ1, **ROS**: Reactive oxygen species**, SARS-CoV-2:** Severe acute respiratory syndrome coronavirus 2, **SLE**: Systemic lupus erythematosus, **SRD5A2**: Steroid 5 alpha reductase alpha polypeptide 2, **STZ**: Streptozotocin, **TGF-α:** Transforming growth factor alpha, **TLR-2**: Toll-like receptor 2 **TLR-4:** Toll-like receptor 4, **TNF-α**: Tumor necrosis factor-α, **VAS**: Visual analogue scale, **VEGF**: Vascular endothelial growth factor

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