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# Therapeutic Effects of Bee Venom on Rheumatoid Arthritis

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# Therapeutic Effects of Bee Venom on Rheumatoid Arthritis

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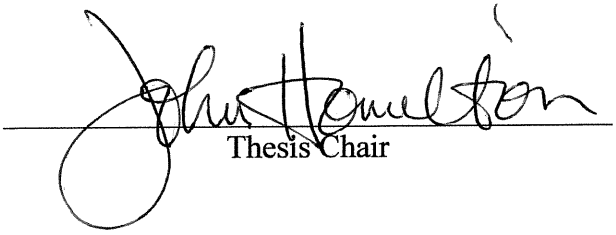
Thesis Committee:



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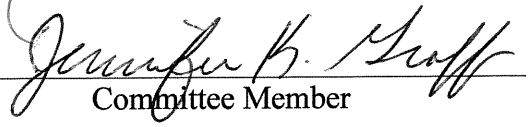

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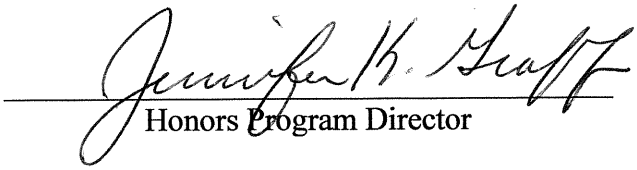

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Many beehive products such as propolis, Manuka honey, bee pollen, and royal jelly have been used by people due to their popular known health benefits. A novel bee product, bee venom, has demonstrated effective in treating various debilitating conditions, and thus bee venom therapy has been the subject of scientific studies to quantify its medical efficacy. Although bee venom has been used for millennia in Eastern medicine, modern bee venom therapy (BVT) originated in Europe in the 19th century, but was brought to the U.S. by Dr. Bodog Beck after World War I. The treatment became more widespread in the West as the 20th century progressed (Cooney et al, 2011). There are currently various application methods with the most popular being apipuncture (acupuncture with bee venom where needles are initially dipped into the venom before applied to the patient) and bee venom injections (Mohammadi et al, 2015). The multiple beneficial effects of bee venom include increased blood circulation, stimulation of the pituitary-cortical system, and reduced pain and inflammation (Mohammadi et al, 2015). These responses help treat the symptoms of many diseases and chronic inflammatory conditions. With additional research, the use of bee venom can be included as a means to safely alleviate the symptoms of several autoimmune diseases, especially rheumatoid arthritis (RA).

Rheumatoid arthritis is a progressive and chronic inflammatory disease that affects the joints and deteriorates other systems such as skin, blood vessels, heart, and eyes. This autoimmune disorder causes the body to attack the synovium lining of the joints which causes weakening and stretching of the ligaments and tendons surrounding the joints (Woo-Ram et al, 2009). As a result, the synovium grows thicker causing the joints to lose their alignment and shape. This is followed by severe inflammation that results in bone erosion causing chronic pain. Although the exact trigger of this process is unknown, doctors suspect underlying genetics that don't necessarily cause rheumatoid arthritis but make patients more susceptible to

environmental agents such as carcinogens, silica, bacteria and viruses that can trigger it (Deok-Sang et al, 2015). Symptoms include joint and blood vessel swelling, pain, morning stiffness, lumps and discoloration under the skin, dryness in eyes and mouth, anemia, and various others. Usually occurring in more than one joint, not limited to any particular joints, Rheumatoid Arthritis affects almost 1.5 million people in the United States with three times the number of females affected than males. It begins in women between the ages of 30 and 60, however, it appears in men much later in life (Dongxing et al, 2015). Current medication has been helpful in treating to cooperate with rheumatoid arthritis, however, contain many harmful side effects such as stomach problems, internal bleeding, liver damage, and severe toxicity (Dongxing, et al, 2015). Current medication also does not provide a permanent cure. Bee venom therapy shows promising results as an alternative treatment option which might lead to a closer step to an effective treatment with further research.

It is commonly known that when a bee stings and delivers its venom, it results in the bee's death. This is due to the abdominal rupture which occurs when the bee is unable to pull out its barbed stinger. Thus it leaves behind its stinger, parts of its abdominal and digestive tract along with the associated muscles and nerves (Smart et al. 2017). Therefore, extracting bee venom without causing mortality requires a special machine, a venom collection frame, which is designed to stimulate venom ejection without harm to the bee. The collection frame is composed of plates and a pulse generator which delivers minor electrical shocks, or sometimes generate specific sound frequencies which stimulate a sting reflex (Abrantes, et al 2017). An alarm odor from the venom that has been already ejected onto the glass sheet irritates neighboring bees to sting the glass and release additional venom. Because the bee is not stinging a penetrable surface, the stinger remains intact with the abdomen, thus there is no harm to the bee. Upon

completion, the bees are shaken off the device and the venom is scraped into a container to be immediately taken into a laboratory for processing and storage (Abrantes, et al 2017). Depending on the country, there are different rules and regulations around the storage of bee venom. For example, in Brazil, the moisture and protein contents are specified as well as the packaging type in order to preserve the efficacy and purity of the venom (Abrantes, et al 2017).

Bee venom therapy is an alternative to current conventional treatments for Rheumatoid Arthritis which include DMARDs, disease-modifying antirheumatic drugs such as leflunomide, hydroxychloroquine, methotrexate, sulfasalazine (Jae-Dong et al, 2005). Non-steroidal anti-inflammatory agents (NSAIDs) and corticosteroids are also commonly used for treatment. Bee Venom is produced by both worker bees and queen bees is composed of Mellitin, Apamin, Adolapin, enzymes such as Phospholipase $A_2$ and hyluronidase (Jae-Dong et al, 2005). It is because of this very unique peptide and protein composition of bee venom that individuals allergic to yellow jackets, wasps, and insects can still use bee venom as a treatment option (Jung et al, 2017).

To start with, Melittin is composed of 26 amino acids comprising 50% of bee venom. This powerful cell lytic agent contains anti-inflammatory properties (Darwish et al. 2013). In a study conducted by Park and colleagues, researchers investigated the molecular mechanisms of the anti-arthritic effects of BV compared to Melittin. In order to do this, researchers looked into the different components of RA inflammation: Prostaglandin ($PGE_2$) production, Nitric Oxide (NO) production, expressions of inflammatory genes (COX-2, iNOS, $cPLA_2$), and NF-kB DNA binding. By observing the synoviocytes of Complete Freund's Adjuvants (CFA) induced RA rat models, researchers found the average amount of $PGE_2$ production to be 5400 CPM (counts per minute) and the average NO generation to be 10 μM.

After being treated with BV, the $PGE_2$ production decreased to by nearly 50% to 3000 CPM and the NO generation decreased to 3.9 μM (Park et al. 2004). The synoviocytes treated with isolated Melittin also showed that $PGE_2$ production decreased to 3200 CPM and the NO generation to 5 μM (Park et al. 2004). This shows that Melittin does play a role in decreasing production of $PGE_2$ and NO but the BV has a greater effect in decreasing these factors. Researchers concluded that the other components of BV allow this greater result by possible synergistic interaction of Melittin and the other components (adolapin, apamin, etc.) (Park et al. 2004).

In another experiment, researchers looked at the effect of isolated Melittin compared to complete BV on the expression of inflammatory genes: cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and cytosolic phospholipase $A_2$ ($cPLA_2$). These inflammatory genes are highly expressed in the case of RA due to the chronic inflammation. By using western blotting, researchers found that the expression of COX-2 decreased by 70% with BV and 80% with Melittin (Guastadisegni et al. 2002). iNOS expression decreased by 60% with BV and 96% with Melittin while $cPLA_2$ expression decreased by 30% with BV and 70% with Melittin. (This last result was probably due to BV already containing $PLA_2$ as one of its components. Research is still being conducted on what the exact role $PLA_2$ has in the anti-inflammatory process (Guastadisegni et al. 2002). The transcription of these inflammatory genes is regulated by protein Nuclear Factor Kappa B (NF-kB). To understand the role Melittin plays in the molecular mechanism of decreasing the expression of those genes, researchers experimented with exposing cells to tumor necrosis factor alpha (TNF-α) which activates NF-kB. They found that Melittin reduces TNF-α activity by 57% (Guastadisegni et al. 2002). They concluded that Melittin results in anti-inflammatory actions through a cascade of events starting with inhibiting TNF-α, which

can then no longer activate NF-kB, which in turn can no longer regulate the transcription of the inflammatory genes (Guastadisegni et al. 2002).

Another molecular mechanism of Melittin affecting NF-kB activity is examined when looking into the NF-kB DNA binding activity in terms of its various protein subunits. In this experiment, researchers used an electrophoretic mobility shift assay (EMSA) on a nuclear extract of synoviocytes. They found that Melittin significantly inhibited NF-kB subunits p50, p-IkB, and IkB based on their presence in intensity on the assay (Xie et al. 1999). Researchers concluded that by inhibiting these subunits, NF-kB DNA binding activity is decreased and therefore there is decreased expression of the inflammatory genes triggered by NF-kB (Xie et al. 1999).

Phospholipase $A_2$ (PLA 2) makes up to10-12% of bee venom and is the main component responsible for causing anaphylactic shock in sensitive individuals (Putz et al, 2006). However, $PLA_2$ can stimulate production of regulatory T cells which play a role in suppressing inflammation and regulating immune response. This is illustrated in an inflammation-induced rat model with three different treatments: 1% ovalbumin, control, and 1% ovalbumin with $PLA_2$. Compared to the control, there was an increased production (the highest amount out of the three treatments) of regulatory T cells by 12% in the rats treated with ovalbumin and $PLA_2$ (Park et al. 2016). Regulatory T cells use additional suppressive strategies to resolve inflammation and limit tissue damage. The proposed mechanism model of regulatory T cell stimulation is that $PLA_2$ binds to receptor CD206 of dendritic cells which increases COX-2 expression and stimulates production of $PGE_2$ (initially increasing inflammation in the short term). $PGE_2$ binds receptor EP2 promotes regulatory T cell differentiation. This Treg cell secretes cytokine interleukin 10 (IL-10) which then suppresses inflammatory helper T cells (TH-

1, TH2, TH17) (Lee et al. 2017). Further research is being conducted to better understand the molecular mechanism of $PLA_2$, and whether it is better to include or isolate it out of BV for the treatment of RA.

Adolapin is a basic polypeptide which makes up 2% of bee venom and is believed to also have anti-inflammatory properties. This characteristic is demonstrated in a study where rats with induced edema were treated with saline and 20μg/ml of adolapin followed by measuring the degree of inflammation of their hind paw using a tool called differential volumeter. Results showed that the rats treated with the adolapin showed a 70% decrease in inflammation compared to the control (Shkenderov et al. 1997). Adolapin shows another anti-inflammatory effect by inhibiting cyclooxygenase (COX) of the microsomes of rat spleens in a study conducted by Shkenderov and colleagues. COX is responsible for the formation of prostaglandins that stimulate inflammation (Woo-Ram et al, 2009). In this study, researchers pre-incubated microsomes with 1.5μg/ml Adolapin and noted the COX activity of the microsomes by spectrophotometer assay. Shkenderov and colleagues found that microsomes treated with Adolapin illustrated inhibition of COX activity by 85% (Shkrenderov et al. 1997). By inhibiting cyclooxygenase, the prostaglandins involved in the chronic inflammation are also subsequently inhibited (Woo-Ram et al, 2009). Adolapin may also show analgesic properties. In a study conducted by Michailova and colleagues, they measured peritoneal contractions in groups of mice after intraperitoneal injections of one group receiving 0.1ml of 3% acetic acid and another group receiving 0.3 μg prostaglandin E1 in 0.1ml of saline. These non-control groups were injected with either 20 or 50 μg/kg adolapin 30 minutes before the irritants. By observing the peritoneal contractions, they noted a 60 to 80% inhibition of irritation in the adolapin treated groups (Michailova et al. 2002). Researchers concluded that this could be due to possible

analgesic effect more research is needed and being conducted to better understand the exact anti-inflammatory role and analgesic mechanism of Adolapin in treating RA.

Hyaluronidase and Apamin are the two final discussed components of BV. Although their therapeutic properties are suggested, there is not as much research conducted in these protein compositions as the ones already mentioned. Hyaluronidase makes 2% of bee venom and is responsible for catalyzing hyaluronic acid via a hydrolysis reaction (Masuda et al, 2010). This enables the bee venom to embed itself into tissues by increasing cellular permeability and increasing blood circulation. Apamin is a peptide of 18 amino acids and comprises 3% of bee venom (Gauldie et al, 2006). This peptide is responsible for blocking potassium channels activated by calcium ions, which normally contributes to the painful sensation felt by an individual stung by a bee. However, in repeated dosages and treatments of bee venom therapy, Apamin was shown to stimulate the central nervous system (CNS) and the peripheral nervous system (PNS) so that central and peripheral pain threshold increased (Woo-Ram et al, 2009). This change consequently decreased the sensation of pain for individuals with Rheumatoid Arthritis. This anti-inflammatory biologically active peptide also preserves erythrocytes, stimulates cortisone release, and increases the body's defense capacity (Woo-Ram et al, 2009). Research is still being conducted and the future research will address actual animal models to better understand the exact role and molecular mechanism of these two proteins.

In the case of treating Rheumatoid Arthritis (RA), the beneficial effects of BV reduce stiffness and pain in joints and contribute to other pharmacological actions through immunomodulation. BV has been shown to block the synthesis of pro-inflammatory substances such as cytokinine, TNF-2 and the COX-2 enzyme. BV also inhibits the spread of rheumatoid synovial cells (Darwish et al, 2013). One study indicated that BV directly counteracts the

inflammatory effects of cigarette smoke condensate (CSC) in a subject afflicted with RA – confirming the anti-inflammatory effects of BV (Darwish et al, 2013). Another study has demonstrated that "BV injection into the Zusanli acupoint has both anti-inflammatory and anti-nociceptive effects on Freund's adjuvant-induced arthritis in rats" (Hyun-Woo et al, 2005). The healing properties of BVT have been scientifically demonstrated. Based on the strength of the medical evidence, it can be said that a combined application of traditional medication and bee venom therapy is more effective than the simple use of medication to alleviate rheumatoid arthritis (Hyun-Woo et al, 2005). This was shown in a study where adjuvant induced arthritis in rats was treated with either methotrexate alone or a combination of methotrexate and bee venom. Not only was the latter combination more effective in reducing arthritic inflammation, it also reduced the hepatoxicity of the methotrexate by one third (Young-Bae et al, 2002). Given the long term use of BVT in both traditional Eastern medicine and now in the West in the last hundred years, the positive benefits need to be further explored and systematized into new treatment plans to benefit the sufferers of RA (Hyun-Woo et al, 2005).

BVT can also provide better results in combination therapy with current medication. In a study conducted by Darwish and colleagues, 50 male wistar rats *(Rattus rattus)* were injected with Complete Freund's Adjuvants (CFA) which induced RA (Darwish et al. 2013). They were divided into 5 groups: one control group (only CFA), one group was given saline, one group was given methotrexate (standard DMARD), one group was given BV injections, and the last group was given both methotrexate and BV injections (Darwish et al. 2013). After 21 days of observation following the various treatments, results were measured in terms of the amount of cytokine TNF-α and expression of the NF-kb signal pathway (both play major role in systemic inflammation therefore expressed in all rats with CFA). Methotrexate treated rats were shown to

decrease expression of NF-kb by 30% but there was not a significant reduction in TNF-α. Rats treated with BV injections showed a 50% reduction in expression of NF-kb and a 30% reduction in TNF-α (Darwish et al. 2013). However, the combined treatment of both methotrexate and BV injections showed a 67% reduction in TNF-α and 71% reduction in NF-kb expression. In conclusion, although BV injections reduced swelling more than the methotrexate treatment, combining both treatments, BV injections and methotrexate, shows a combined greater beneficial effect in reducing the factors of inflammation (Darwish et al. 2013).

Rheumatoid arthritis is a debilitating autoimmune disease with millions of sufferers across the globe (Cooney et al, 2011). Traditional treatments with steroids, immunosuppressant and anti-inflammatory drugs can offer only temporary relief from the pain, while the use of the drugs themselves can have negative side-effects which cause their own health issues. The exploration of BVT by Western scientific methods is a beneficial step in proving the efficacy of this traditionally Eastern medical treatment. With the clear anti-inflammatory benefits of a measured systematic treatment plan without the harmful side-effects of current medications, the applications of BVT seem promising in creating the means to safely alleviate the symptoms of RA as well as many other autoimmune diseases.

Altogether more research needs to be done on the individual BV components to determine which one is more beneficial for the treatment of RA. Whether it is primarily Melittin or the addition of the other isolated peptides such as Adolapin or phospholipase A2 which promise to provide the most effective means for alleviating arthritis symptoms still remains to be seen. The full mechanisms of BVT require further studies and trials in order to be fully understood and applied with the greatest efficacy.

Citations

Abrantes, Allyson, et al. "Honeybee Venom: Influence of Collection on Quality and Cytotoxicity." ["Veneno de abelha: influência da coleta em qualidade e citotoxicidade"]. Ciência Rural, vol. 47, no. 10, Oct. 2017, pp. 1-4. EBSCOhost, doi:10.1590/010

Cooney, Jennifer, et al. "Benefits of Exercise along with Treatments in Rheumatoid Arthritis," Journal of Aging Research, vol. 2011, Article ID 681640, 14 pages, 2011. doi:10.4061/2011/681640

Darwish, Samar F., et al. "Targeting TNF-α and NF-κB Activation by Bee Venom: Role in Suppressing Adjuvant Induced Arthritis and Methotrexate Hepatotoxicity in Rats." PLoS ONE, vol. 8, no. 11, 2013, doi:10.1371/journal.pone.0079284.

Deok-Sang, Hwang, et al. "Therapeutic Effects of Bee Venom on Immunological and Neurological Diseases." Toxins, Vol 7, Iss 7, Pp 2413-2421 (2015), no. 7, 2015, p. 2413. EBSCOhost, doi:10.3390/toxins7072413.

Dongxing, Li, et al. "Analgesic Effects of Bee Venom Derived Phospholipase A2 in a Mouse Model of Oxaliplatin-Induced Neuropathic Pain." Toxins, vol. 7, no. 7, 2015, pp. 2422–2434., doi:10.3390/toxins7072422.

Fortunato Abrantes, Allyson, et al. "Honeybee Venom: Influence of Collection on Quality and Cytotoxicity." ["Veneno de abelha: influência da coleta em qualidade e citotoxicidade"]. Ciência Rural, vol. 47, no. 10, Oct. 2017, pp. 1-4. EBSCOhost, doi:10.1590/010.

Gauldie, Jack, et al. "The Peptide Components of Bee Venom." European Journal of Biochemistry, vol. 61, no. 2, Jan 2006 Part 2, pp. 369-376. EBSCOhost, doi:10.3345/components9879

Guastadisegni, Cecilia, et al. "Modulation of $PGE_2$ and TNF-α by Nitric Oxide in Resting and

LPS-Activated Raw 264.7.” *Cytokine*, vol. 19, no. 4, 8 July 2002, pp. 175–180., doi:10.1006/cyto.2002.1955.

Hyun-Woo, Kim, et al. “Antinociceptive Effects Of Bee Venom Acupuncture (Apipuncture) In Rodent Animal Models: A Comparative Study Of Acupoint Versus Non-Acupoint Stimulation.” Acupuncture & Electro-Therapeutics Research, vol. 26, no. 1, Jan. 2001, pp. 59–68., doi:10.3727/036012901816356054.

Jae-Dong, Lee, et al. "An Overview of Bee Venom Acupuncture in the Treatment of Arthritis." Evidence-Based Complementary and Alternative Medicine, Vol 2, Iss 1, Pp 79-84 (2005), no. 1, 2005, p. 79. EBSCOhost, doi:10.1093/ecam/neh070.

Jung, Kyung-Hwa et al. “Protective Effects of Intratracheally-Administered Bee Venom Phospholipase A2 on Ovalbumin-Induced Allergic Asthma in Mice.” Ed. Andreimar M. Soares. Toxins 8.10 (2016): 269. PMC. Web. 13 Oct. 2017.

Lee, Gihyun, et al. “Bee Venom Phospholipase A2: Yesterday’s Enemy Becomes Today’s Friend.” Ed. Glenn F. King. Toxins 8.2 (2016): 48. PMC. Web. 13 Oct. 2017.

Masuda, Seiko, et al. “Various secretory phospholipase A2 enzymes are expressed in rheumatoid arthritis and augment prostaglandin production in cultured synovial cells.” FEBS Journal, vol. 272, no. 3, Oct. 2010, pp. 655–672., doi:10.1111/j.1742-4658.2004.04489.x.

Mohammadi, Ebrahim, et al. "Immunomodulatory Effects of Bee Venom in Human Synovial Fibroblast Cell Line." Iranian Journal of Pharmaceutical Research, vol. 14, no. 1, Jan. 2015, p. 313. EBSCOhost, doi:10.8237/s0056-345

Park, Soojin, et al. “Bee venom phospholipase A2 suppresses allergic airway inflammation in an

ovalbumin-Induced asthma model through the induction of regulatory T cells." Immunity, Inflammation and Disease, vol. 3, no. 4, 9 Feb. 2015, pp. 386–397., doi:10.1002/iid3.76.

Park, Hye Ji, et al. "Antiarthritic effect of bee venom: Inhibition of inflammation mediator generation by suppression of NF-?B through interaction with the p50 subunit." *Arthritis & Rheumatism*, vol. 50, no. 11, 4 Nov. 2004, pp. 3504–3515., doi:10.1002/art.20626.

Park, Yeon Cheol, et al. "Long-Term Effectiveness of Bee Venom Acupuncture and Physiotherapy in the Treatment of Adhesive Capsulitis: A One-Year Follow-Up Analysis of a Previous Randomized Controlled Trial." Journal of Alternative & Complementary Medicine, vol. 20, no. 12, Dec. 2014, p. 919. EBSCOhost, doi:10.1089/acm.2014.0220.

Putz, Thomas, et al. "Bee venom secretory phospholipase A2 and phosphatidylinositol-Homologues cooperatively disrupt membrane integrity, abrogate signal transduction and inhibit proliferation of renal cancer cells." Cancer Immunology, Immunotherapy, vol. 56, no. 5, Jan. 2006, pp. 627–640., doi:10.1007/s00262-006-0220-0.

Smart, Matthew et al. "Linking Measures of Colony and Individual Honey Bee Health to Survival among Apiaries Exposed to Varying Agricultural Land Use." Ed. Raul Narciso Carvalho Guedes. *PLoS ONE* 11.3 (2016): e0152685. *PMC*. Web. 29 Nov. 2017.

Woo-Ram, Lee, et al. "The Protective Effect of Bee Venom on Fibrosis Causing Inflammatory Diseases." Toxins, vol. 7, no. 12, 2015, pp. 4758–4772., doi:10.3390/toxins7114758.

Xie, QW, et al. "Role of the transcription factor, NF-κB, in the expression of inducible nitric oxide synthase in helicobacter pylori gastritis." *Free Radical Biology and Medicine*, vol. 23, no. 27, 6 Mar. 1999, pp. 303–317., doi:10.1016/s0891-5849(99)90692-5.

Young-Bae, Kwon, et al. "The Water-Soluble Fraction of Bee Venom Produces Antinociceptive

and Anti-Inflammatory Effects on Rheumatoid Arthritis in Rats." Life Sciences, vol. 71, no. 2, May 2002, pp. 191-204. EBSCOhost, doi:10.1016/S0024-3205(02)01617-X.