https://dx.doi.org/10.4314/ejppri.v7i2.8

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (2): 244–254

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Impact of bee venom acupuncture on the blood cells and some biochemical parameters in systemic lupus erythematosus patients

Mona, A. A. Amer[1]; Noran, O. El-Azizi[2]; Emad A. Nafea[3]; Mohamed, S. Younis[3] and Ahmed, S. Abou Zeid[1]

[1]Faculty of Science, Ain Shams University, Cairo, Egypt.
[2]Faculty of Medicine, Ain Shams University, Cairo, Egypt.
[3]Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt.

ARTICLE INFO
Article History
Received: 23/4/2024
Accepted: 24/6/2024

Keywords

Lupus, honeybee venom, acupuncture, blood chemistry and biochemical parameters.

Abstract

Patients with systemic lupus erythematosus (SLE) were allocated to two main groups (A) and (B) with the same disease activity, each group included fifteen patients, and their ages ranged between 21-60 years old. In addition to the conventional treatment Group (A) was treated with Bee venom acupuncture (BVA) twice a week for three months, while patients of group (B) were kept on the conventional treatment only. Obtained results indicated that the serum levels of group (A) were significantly increased for hemoglobin (Hb) and complementary 3.The values for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Protein/Creatinine (P/C) ratio, 24 hrs. urinary protein, urinary pus cells (H.P.F), urinary protein and complement 3 (C3) were significantly decreased ($p<0.05$). No significant differences were recorded for total leucocytic count (TLC), platelets (PLT), serum Creatinine (S Cr.), urinary red blood cells (RBCs) and complement 4 (C4), where they remained unchanged after treatment. The results showed a statistically significant difference between the two SLE groups regarding DNA titer and ANA titer as P-Value < 0.05.

Introduction

Since ancient times, people have used bee products such a honey, pollen, propolis, bee wax, royal jelly, and venom as food and medicine. Because it contains active ingredients such as peptides, proteins, enzymes, bioactive amines, phospholipids, and sugars, bee venom is particularly significant among them. It was used to treat skin conditions, rheumatism, arthritis, and back discomfort. Today, it is used to treat a variety of conditions, including cancer, neurological disorders, Parkinson's disease, tissue stiffening, gout, arthritis, ocular, skin, liver, cardiovascular, ulcerative, breast, cancer, eczema, epilepsy, sinusitis, cholesterol, and asthma. It is also used to treat wounds, vascular occlusion, musculoskeletal problems, flu infections, and in cosmetics. In addition to its antibacterial, antiviral, anti-inflammatory, antidiabetic, anti-arthritis, antimutagenic, anticancer, antinociceptive, neuroprotective, and

radioprotective properties, this substance also possesses anti-inflammatory analgesic, anti-acne, anti-viral, anti-aging, wound-healing, anti-hyperglycemic, and anti-asthmatic properties (Abaci and Orhan, 2022 and Yapici *et al*., 2023). The Cause of SLE is unclear, there may be a genetic trigger coupled with an environmental trigger, resulting in defects of the immune system. Factors associated with SLE is vitamin D deficiency, female sex hormones, sunlight and smoking (Frieri and Stampfl, 2016).

Common symptoms vary among people and may be mild to severe. Symptoms include painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red malar rash which is most commonly on the face. Women are affected about nine times more often than men and the symptoms associated with each sex are different (Lisnevskaia *et al*., 2014). Since acupuncture points are more effective than non-acupoints, a number of studies have revealed that the effects of Bee Venom Acupuncture (BVA) vary depending on the injection site. Therefore, only when BV is injected into a particular acupoint, it will exhibit its anti-nociceptive activity (Kwon *et al*., 2001; Seo *et al*., 2003 and Kim *et al*., 2003). Additionally, BVA stimulates both pharmacological and mechanical functions through acupuncture stimulation; it is thought that BVA has been suggested as an alternative treatment method to needle acupuncture (Lee *et al*., 2005). Numerous research' findings support the application of this therapy as a clinical treatment for a wide range of human illnesses (Lee *et al*., 2006). For this reason, this study was designed in accordance with traditional Chinese medicine (TCM), which states that the bee sting must occur at a specific acupoint.

The aim of the present study is to evaluate the efficacy of bee venom acupuncture on blood cells and some biochemical parameters in Lupus erythematosus patients.

Material and patients

1. Bee venom:

Ten colonies of healthy first hybrid (F1) Carniolian honeybees, *Apis mellifera carnica,* were reared in the apiary yard at the Apiculture Research Department, Plant Protection Research Institute, Agricultural Research Center at Dokki, Giza, Egypt. The routine work for keeping and developing of the colonies was carried out during the experimental period. The venom of the adult honeybee workers *Apis mellifera* was used to treat SLE patients, through live bee stinging, from late spring to the end of summer, where the bee hives were strong and at biological balance. Adult honeybee workers who are 21 days in age were collected from the hives (Not young workers), because they have a complete venom component (Schumacher *et al*., 1989).

2. Patients with Systemic Lupus Erythematosus (SLE):

Patients with SLE were allocated to two main groups A and B with the same disease activity, from mild to severe activity, each group included fifteen patients and ages ranged between 21-60 years old from the outpatient clinic and the inpatients of department of Rheumatology at the Ministry of Health hospitals. Group A of the patients with SLE was treated with conventional drug therapy for three months, in addition to bee venom therapy (BVT). Three months is the minimum period needed for the body's systems to react with the new therapeutic material, especially in the cases of chronic inflammatory diseases, such as SLE (Ozdemir *et al*., 2011). Group B (Control group) SLE patients were on the conventional treatment only. Group A was treated with BV

twice a week for 3 months (Total of 24 sessions), after subjecting to the allergy test. The treatment started with one sting in the first session, two stings in the second session after two days, and two stings were gradually increased every session until reaching twenty stings per session at twenty acupoints of the body, just one sting at each point (Figure 1), through which SLE could be controlled, and according to the order suggested by Lee *et al.* (2011).

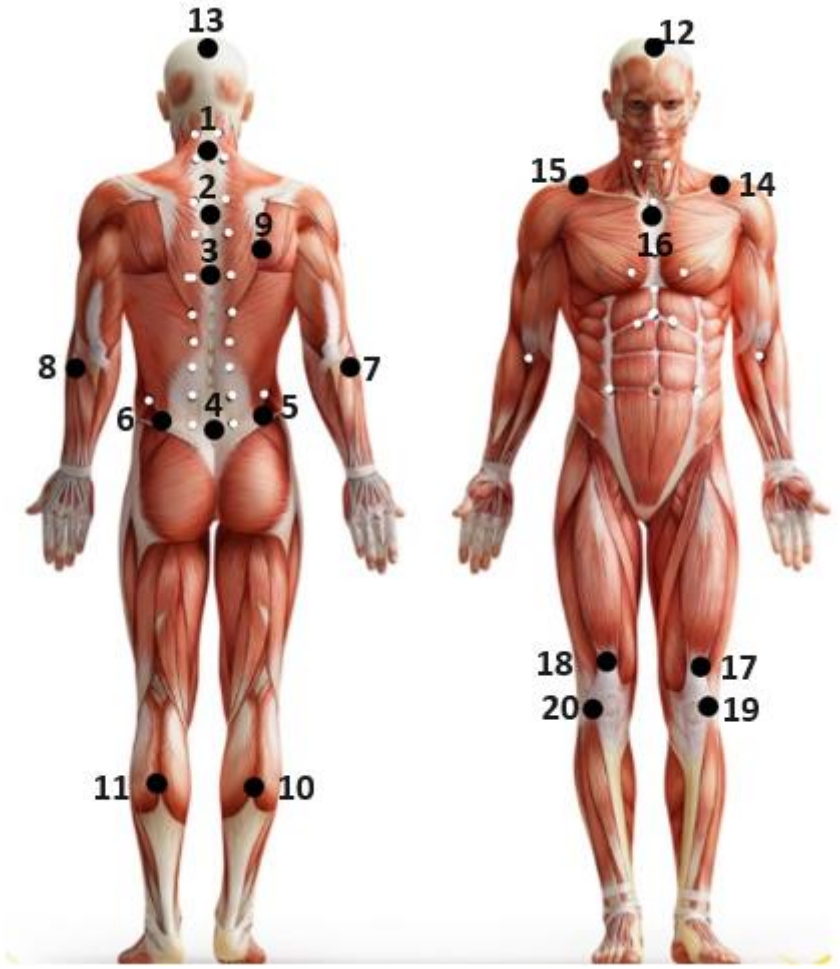

Figure (1): The acupoints (black points) specialized for controlling SLE disease through bee venom treatment BVT (Lee et al., 2015). The stinging starts from the acupuncture point No. 1, then No. 2, and continues in order until reaching the point No. 20 is reached.

3. Laboratory investigations:

According to Lisnevskaia *et al*. (2014**),** laboratory investigations were done for the two groups of patients before and after treatment including:

3.1. Complete blood count (CBC) was done by automated count technology regarding total leucocytic count (TLC), hemoglobin (Hb.) and platelets (PLT).

3.2. Erythrocyte sedimentation rate (ESR) in the first hour, estimated by Westergren method recorded in mm/hr.

3.3. C-reactive protein (CRP) using the method of enzyme linked immunosorbent assay (ELISA).

3.4. Kidney function serum creatinine (S Cr.) was done by calorimetric method.

3.5. Protein/Creatinine ratio (p/c) was done by kinetic method.

3.6. 24hrs. urinary protein was done by kinetic method.

3.7. Urine analysis by manual and microscopic technique regarding urinary red blood cells (RBCs), pus cells (WBCs), protein (Albumin) and casts.

3.8. Complement 3 (C3) and Complement 4 (C4) by latex agglutination test.

3.9. Anti DNA (Anti Deoxyribonucleic Acid) titer and ANA (Anti- Nuclear Antibodies) by ELISA.

4. Statistical analysis:

The collected data was revised, coded, tabulated and analyzed using the Statistical Package for Social Science (SPSS 25).

Results and discussion

This study included 30 SLE female patients, 30% of them are single and 70% are married. The age of patients ranged from 21 to 60 years with a mean age of 36.90±10.4 years, and the disease duration range was from 2-27 years with a mean of 11.30±6.33 years. The medical treatment duration ranged from 2-27 years with a mean of 10.97±6.15. None of our studied SLE patients suffered from diabetes mellites, while 76.7% of them suffered from blood hypertension.

All studied SLE patients suffered from malar rash, photosensitivity, oral ulcers, alopecia, neuropsychiatric and fever, 93.3% complained of arthritis, 86.7% had renal (lupus nephritis) and gastrointestinal tract affection, 83.3% complained of body rash, 80% from eye problems and 76.7% from hypertension symptoms, while 73.3% complained from pulmonary affection, 63.3% from increased appetite and weight and 36.7% from decreased appetite and weight. 20% of patients had renal biopsy. The least manifestations were arthralgia (6.7 %) and cardiac affection (33.3 %).

The tested biochemical parameters are shown in Table (1). Means ± SE for serum levels of the treated group were significantly increased for hemoglobin (Hb.), where it was 8.95 ± 0.91 and 10.91 ± 0.61 before and after treatment, respectively ($p<0.001$) and for complement 3, where it was 95 (90-98) and 86 (55-95) before and after treatment, respectively ($p<0.001$). The values for erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), Protein/Creatinine (P/C) ratio, 24hrs urinary protein, urinary pus cells (H.P.F), urinary protein and complement 3 (C3) were significantly decreased ($p<0.05$). No significant differences were recorded for total leucocytic count (TLC), platelets (PLT), serum Creatinine (S Cr.), urinary red blood cells (RBCs) and complement 4 (C4), where they remained unchanged.

Table (2) shows a statistically significant difference between the two SLE groups regarding DNA titer and ANA titer as P-Value < 0.05. RNA titer was recorded in all patients of the two groups before treatment. After treatment with BV it was recorded in only 20% of the patients, while it was recorded in all patients of the control (untreated) group with a significant difference between the two groups ($p<0.001$). Anti-dsDNA titer was positive in all 30 patients before treatments. After treatment there was a significant difference between the two group, where it was positive in only 40% of patients of the treated group, while it remained positive in 100% of untreated group patients ($p<0.001$).

Table (1): The laboratory investigations between the two studied SLE groups.

Laboratory investigations		Group		Test of significance	
		Group A (Cases)	Group B (Controls)		
		Median (IQR) N (%)	Median (IQR) N (%)	p-Value	Sig.
Total leucocytic count (TLC) (10^3/uL)	Before	5.4 (2.8 - 6.9)	5.5 (2.9 - 6.6)	0.740*	NS
	After	5.2 (4.6 - 5.5)	5.5 (2.9 - 6.7)	0.493*	NS
Wilcoxon signed test		0.776 (NS)	0.952 (NS)		
Hemoglobin (Hb.) (g/dl)	Before	8.95 ± 0.91	8.88 ± 0.91	0.842**	NS
	After	10.91 ± 0.61	9.2 ± 1	<0.001**	S
Paired t-test		<0.001 (S)	0.019 (S)		
Platelets (PLT) (10^3/uL)	Before	236.2 ± 93.38	233.93 ± 92.8	0.947**	NS
	After	226.4 ± 70.53	236.73 ± 88.42	0.726**	NS
Paired t-test		0.43 (NS)	0.285 (NS)		
Erythrocyte sedimentation rate (ESR) (mm/hour)	Before	45 (30 - 70)	45 (35 - 75)	0.868*	NS
	After	20 (15 - 30)	45 (30 - 62)	0.003*	S
Wilcoxon signed test		0.001 (S)	0.004 (S)		
C- reactive protein (CRP) (mg/l)	Before	58 (24 - 80)	58 (20 - 64)	0.786*	NS
	After	11 (6 - 15.5)	48 (12 - 64)	0.003*	S
Wilcoxon signed test		0.001 (S)	0.04 (S)		
Serum Creatinine (S Cr.) (mg/dl)	Before	1.1 (0.8 - 1.5)	1.1 (0.8 - 1.5)	0.917*	NS
	After	1 (0.8 - 1.1)	1.1 (0.8 - 1.4)	0.379*	NS
Wilcoxon signed test		0.007 (S)	0.029 (S)		
Protein/Creatinine (P/C) ratio (mg pro/g cr.)	Before	4.5 (3.7 - 5.2)	4.9 (3.7 - 5.3)	0.739*	NS
	After	2.1 (0.2 - 3.1)	4.4 (3.5 - 5.2)	<0.001*	S
Wilcoxon signed test		0.001 (S)	0.005 (S)		
24hr urinary protein (mg/day)	Before	2.3 (0.43 - 3.1)	2.4 (0.43 - 3.3)	0.742*	NS
	After	1 (0.2 - 1.1)	2.3 (0.3 - 3.1)	0.029*	S
Wilcoxon signed test		0.011 (S)	0.004 (S)		
Urinary red blood cells (RBCs) (H.P.F)	Before	4 (3 - 7)	4 (3 - 7)	0.735*	NS
	After	3 (2 - 4)	4 (2 - 4)	0.559*	NS
Wilcoxon signed test		0.063 (NS)	0.026 (S)		
Urinary pus cells (H.P.F)	Before	20 (6 - 45)	25 (8 - 45)	0.867*	NS
	After	8 (4 - 10)	20 (8 - 35)	0.023*	S
Wilcoxon signed test		0.003 (S)	0.028 (S)		
Urinary protein (pre)	0	6 (40%)	5 (33.33%)	0.796**	NS
	1	6 (40%)	8 (53.33%)		
	2	3 (20%)	2 (13.33%)		
Urinary protein (post)	0	14 (93.33%)	6 (40%)	0.005**	S
	1	1 (6.67%)	8 (53.33%)		
	2	0 (0%)	1 (6.67%)		
Marginal Homogeneity test		0.008 (S)	0.157 (NS)		
Complement 3 (C3) (g/l)	Before	86 (55 - 90)	85 (55 - 95)	0.950*	NS
	After	95 (90 - 98)	86 (55 - 95)	0.006*	S
Wilcoxon signed test		0.004 (S)	0.673 (NS)		
Complement 4 (C4) (g/l)	Before	17 (10 - 30)	16 (10 - 30)	0.967*	NS
	After	20 (19 - 30)	16 (10 - 30.5)	0.132*	NS
Wilcoxon signed test		0.018 (S)	0.05 (NS)		

(10^3/uL): 1000/unit liter, (g/dL): grams per deciliter, (mm/hour): millimeters per hour, (mg/l): milligrams per liter, (mg/dl): milligrams per deciliter, (mg pro/g cr.): milligrams protein per grams creatinine, (mg/day): milli gram per day, (H.P.F): high power field, (g/l): grams per liter.

-P>0.05: Non-significant (NS). -P< 0.05: Significant (S). *Mann-Whitney test, **Fisher's Exact test.

Table (2): The immunological investigations between the two studied SLE groups:

Table (2): Shows statistically significant differences between the two SLE groups, cases and controls, regarding DNA titer and ANA titer as P-Value < 0.05.

Immunological investigations		**Group**		**Test of significance**	
		Group A (Cases)	**Group B (Controls)**		
		Median (IQR) N (%)	**Median (IQR) N (%)**	**p-Value**	**Sig.**
DNA titer (pre) (AU/ml)	**Positive**	15 (100%)	15 (100%)		
DNA (post)	**Positive**	6 (40%)	15 (100%)	0.001**	S
McNemar test		0.004 (S)	————		
ANA titer (pre) (AU/ml)	**Positive**	15 (100%)	15 (100%)		
ANA (post)	**Positive**	3 (20%)	15 (100%)	<0.001**	S
McNemar test		<0.001 (S)	————		

***Mann-Whitney test, **Fisher's Exact test.**

DNA: Deoxyribonucleic Acid and ANA: Anti- Nuclear Antibodies. (AU/ml): Arbitrary unit per milliliter.

-P>0.05: Non significant (NS).

-P< 0.05: Significant (S).

Any region of the body can be affected by systemic lupus erythematosus, often known as lupus or SLE, which is an autoimmune disease that affects the connective tissue in the body. Similar to other autoimmune disorders, inflammation and tissue damage are caused by the immune system attacking the body's cells and tissue (William *et al*., 2005). The disease progresses in an unexpected manner, with remissions interspersed with episodes of illness known as flares. Nine times as many women as males have the condition, particularly those who are pregnant or have recently given birth, between the ages of 15 and 35. People of non-European ancestry are also more likely to get the disease (Anisur and David, 2008).

Because of its distinct structure and abundance of advantageous enzymes and peptides, bee venom (BV), which is derived from the venom gland of honeybees (*Apis mellifera*), is thought to be one of the more potent natural supplements (Kim and Song, 2005). Furthermore, BV extract is more than a dietary supplement; numerous studies have examined its potential to enhance animal productivity, feed efficiency, treat and prevent animal and human diseases (Apitherapy), as well as promote better health (Sturm *et al*., 2002; Rabie *et al*., 2018 and Elhanafy *et al*., 2023). In addition to adolapin (Polypeptides), melittin and apamin are the active ingredients in BV that have anti-inflammatory and antibacterial properties (Baqer and Yaseen, 2018). Melittin, the primary active component, stimulates the pituitary and adrenal glands to produce catecholamine and cortisone, two important anti-inflammatory hormones (Sturm *et al*., 2002). Its antimutagenic (Varanda *et al*., 1999), antinociptive (Baek *et al*., 2006), and radioprotective (Garaj-Vrhovac and Gajski, 2009) effects are other bio-effective properties of BV. Its role in boosting immunological responses has not received much attention (Martinello and Mutinelli, 2021).

In the current study, data of the studied laboratory investigations of group (A) received BVT showed a significant increase in hemoglowasconcentration (Hb%) and a significant decrease in the value of

Erythrocyte sedimentation rate (ESR), C- reactive protein (CRP) and Protein/Creatinine (P/C), 24hr urinary protein, urinary pus cells, urinary protein and complement 3 (C3) as P-Value < 0.05, and there were non-significant difference in the results of the total leucocytic count (TLC), platelets (PLT) and complement 4 (C4) as P-Value > 0.05 in group (A) received BVT. These results are in line with those of Mohammed and Hassan, (2019), who observed a statistically significant rise in Hb values in the arthritic group receiving BVT. The study examined the impact of bee venom on various blood and biochemical parameters in male albino rats suffering from arthritis and compared the results with those of prednisolone medication.

Abo-Zaid *et al*. (2023), investigating the impact of bee venom on immunological and hematological parameters in albino rats, noted a marginal rise in red blood cell count in all BV-treated groups relative to the control group. When compared to the control animals, a substantial rise in the red blood cells (RBC), white blood cells (WBC), platelets (PLT) and Hb concentration value was seen with frequent BV treatment (Muhammad *et al*., 2015).

According to Son *et al*. (2007), BV plays a function in stimulating the production of erythrocytes and can enhance blood circulation in micro blood capillaries, as well as the coronary and peripheral circulations. When compared to the control group, Rabie *et al*. (2018) report that a noteworthy rise in hemoglobin concentration was noted in chicks treated with BV. The results obtained are in good agreement with other recent research that has shown that BV affects blood chemistry and biochemical markers. Furthermore, the results of this investigation indicated a non-significant decline in the effect of BV on the platelet count. These findings conflict with those of Mohammed and Hassan (2019), who observed a statistically significant decline in PLT values in the group receiving BV treatment for arthritis. Furthermore, PLA2 from Egyptian honeybees was found to be useful in postponing platelet aggregation and blood clotting (Darwish *et al*., 2021).

Neutrophils are one kind of WBC that is a part of the immune system that defends the body from outside invaders. Essential information about health status is provided by the total WBC count (Kutlu *et al*., 2020). Research demonstrates that via influencing pro-inflammatory cytokines, the immunosuppressive and anti-inflammatory characteristics of BV decreased WBC count. Numerous studies Yousefpoor *et al*. (2022) have shown a dose-dependent rise in WBC count in healthy rats, however Mohammed and Hassan (2019), Darwish *et al*. (2013) and Abbasifard *et al.* (2021) reported that WBC count decreased by BV therapy after producing inflammation in models. Ivas (2011) described this BV effect as the combination of melittin, phospholipase A2, and polypeptide, which collectively account for about 62% of BV. Additionally, their findings demonstrated a large increase in the value of HB and a significant drop in the values of WBCs and PLT. The current data demonstrate a significant drop in urine protein following BV treatment. This is in good agreement with the Lee *et al*. (2011) findings. Using ZB/W F1 female mice that had renal failure, proteinuria, and idiopathic glomerulonephritis, they discovered that the BV-treated group's mean urine protein level had decreased. According to Hwang *et al*. (2015), BV therapy dramatically lowered tubal damage, avoided renal inflammation, and

postponed the onset of proteinuria. Only one case report by Song *et al*. (2009), in Korea, measured levels of C3 and C4 in a female who developed systemic lupus erythematosus (SLE) after she was exposed to bee venom as an alternative medication. In this case report, they did not discuss any relation between C3, C4 and bee venom or development of SLE.

The present study revealed a statistically significant difference between the two SLE groups regarding DNA titer and ANA titer as P-Value < 0.05. Pre BVT, DNA titer and ANA titer were 100% positive among SLE patients (normal range is negative), the results after the treatment with BV showed that; DNA titer was positive only in 6 patients (40%), while ANA titer was detected only in 3 patients (20 %). According to Sharma *et al*. (2023), a need for mandatory admission is a positive antinuclear antibody titer of 1:80 or above, which is part of the most recent clinical criteria put forth by the American College of Rheumatology and the European League Against Rheumatism in 2019. Obtaining an initial ANA titer is necessary. About 95% of patients have extremely sensitive ANA, despite the test's lack of specificity. Although it is not specific, about 95% of patients have extremely sensitive ANA. According to Shamim *et al*. (2022), 78.3% of the patients had positive anti-ds-DNA results, but all of the patients under study had positive ANA by IFA results.

The results revealed that the bee venom therapy showed significant improvements in group A of SLE patients who received bee venom compared to patients who received pharmacotherapy only. It revealed effective treatment for hypertensive, renal functions, alopecia, arthritis, fertility, anemia, hematuria, pyuria, proteinuria, rash, mucosal ulcers, pleurisy, leukopenia, and decreased the number of doses of immunosuppressive and anti-hypertensive drugs.

This study represented an applicable, reliable, safe, promising and highly effective treatment for systemic lupus erythematosus, by honeybee venom therapy. Bee venom (BV) administration was reported to stimulate the function of the immune system, indirectly inducing the adrenal glands to release the body's own cortisol by stimulating the pituitary gland to release ACTH (Natural form of adrenocorticotropic hormone). It is a magical weapon, which is known as a natural anti-inflammatory agent, it has 100 folds of therapeutic strength than hydrocortisone (Chemical form).

It is concluded that both modes of treatment for SLE gave improvement, regarding, disease assessments and quality of life being more evident in bee venom group supported with improved clinical symptoms and investigations. Although Apitherapy is not a curable therapy for SLE, but it can be used to minimize some of the clinical symptoms of SLE, and can be included among programs of SLE therapy.

Finally, bee venom therapy (BVT) can be used to be included among programs of SLE therapy to obtain a magical therapeutic result, without suffering from any side effects.

References

Abaci, N. and Orhan, I. E. (2022): Bee venom and its biological effects. Current Perspectives on Medicinal and Aromatic Plants, 86-105. https://doi.org/10.38093/cupmap.1127949.

Abbasifard, M.; Yousefpoor, Y.; Amani, A. and Arababadi, M. K. (2021): Topical bee venom nano-emulsion ameliorates serum level of endothelin-1 in collagen-induced rheumatoid

arthritis model. Bio Nano Science, 11:810.

Abo-Zaid, M.A.; Yatimi, K. A. and Ismail, A. H. (2023): The role of bee venom on immunological and hematological parameters in albino rats. The Egyptian Journal of Immunology, 30 (2): 11–25.

Anisur, R. and David, A. (2008): Review Article: Systemic Lupus Erythematosus. New England Journal of Medicine, 358: 929–939.

Baek, Y. H.; Huh, J. E.; Lee, J.D. and Park, D. S. (2006): Antinociceptive effect and the mechanism of bee venom acupuncture (Apipuncture) on inflammatory pain in the rat model of collagen-induced arthritis: mediation by a2-Adrenoceptors. Brain Research, 1073: 305-310. https://doi.org/10.1016/j.brainres.2005.12.086.

Baqer, L. K. and Yaseen, R. T. (2018): The effect of whole honeybee venom (*Apis mellifera*) on reducing skin infection of rabbits caused by methicillin resistant staphylococcus aureus: an in vivo study, Journal of Pure Applied Microbiology, 12: 2111-2116. https:// doi.org/10.22207/JPAM.12.4.48.

Darwish, D. A.; Masoud, H. M.; Abdel-Monsef ,M. M., *et al.* (2021): Phospholipase A2 enzyme from the venom of Egyptian honey bee *Apis mellifera lamarckii* with anti-platelet aggregation and anticoagulation activities. Journal of Genetic Engineering and Biotechnology, 19 (1):1-8.

Darwish, S.F.; El-Bakly, W.M.; Arafa, H.M. and El-Demerdash, E. (2013): Targeting TNF-α and NF-κB activation by bee venom: role in suppressing adjuvant induced arthritis and methotrexate hepatotoxicity in rats. *PLoS One.* 8(11):e79284. Doi: 10.1371/journal.pone.0079284.

Elhanafy, A.I.; Mousa, A. M. and Kamal, A. M. (2023): Immunomodulatory, Anti-Inflammatory and Antioxidant Activities of Bee Venom in Doe Rabbits Under High Ambient Temperature. Journal of Animal Health and Production, 11 (1): 82-89.

Frieri, **M. and** Stampfl, H**. (2016):** Systemic lupus erythematosus and atherosclerosis: Review of the literature. Autoimmune Review,15 (1):16-21. Doi: 10.1016/j.autrev.2015.08.007.

Garaj-Vrhovac, V. and Gajski, G. (2009): Evaluation of the cytogenetic status of human lymphocytes after exposure to a high concentration of bee venom in vitro. Archives of Industrial Hygiene and Toxicology, 60:27e34. https://doi.org/10.2478/10004-1254- 60-2009-1896.

Hwang, D.S.; Sun, K.K. and Hyunsu, B. (2015): Therapeutic effects of bee venom on immunological and neurological diseases. Toxins, 7: 2413-2421; Doi:10.3390/toxins7072413.

Ivas, C. (2011): Glycemia and lipidemia variations of the rabbits inoculated with bee venom. Lucrări Ştiinţifice, Seria Zootehnie, 55:19- 23.

Kim, H. W.; Kwon, Y. B.; Ham, T. W.; Roh, D. H.; Yoon, S. Y.; Lee, H. J.; Han, H. J.; Yang I.

S.; Beitz, A. J. and Lee, J. H. (2003): Acupoint stimulation using bee venom attenuates formalin-induced pain behavior and spinal cord fos expression in rats. Journal of Veterinary Medical Sciences, 65(3):349-55.

Kim, K. T. and Song, H. S. (2005): A randomized controlled double blinding study of bee venom acupuncture therapy on sprain of C-spine. The Acupuncture, 22(4):189–195.

Kutlu, H.; Avci, E. and Özyurt, F. (2020): White blood cells detection and classification based on regional convolutional neural networks. Medical Hypotheses,135:109472.

Kwon, Y. B.; Lee, J. D.; Lee, H. J.; Han, H. J.; Mar, W. C.; Kang, S. K.; Beitz, A. J. and Lee, J. H. (2001): Bee venom injection into an acupuncture point reduces arthritis associated edema and nociceptive responses. Pain, 90(3): 271-280.

Lee, D.; Park, H. J.; Chae, Y. and Lim, S. (2005): An overview of bee venom acupuncture in the treatment of Arthritis. Evidence-Based Complementary and Alternative Medicine, 2 (1): 79–84.

Lee, J. Y.; Han, Y. J.; Kim, J. H.; Kim, Y. J. and Kwon, K. R. (2006): Type analysis of pharmacopuncture. Journal of Pharmacopuncture, 9 (3):147–154.

Lee, S. M.; Yang, E. J.; Choi, S. M.; Kim, S. H.; Baek, M. G. and Jiang, J. H. (2011): Effects of bee venom on glutamate-induced toxicity in neuronal and glial cells. Evidence-Based Complementary and Alternative Medicine, (3):368196: pp.11. Doi: 10.1155/2012/368196.

Lisnevskaia, **L.**; Murphy, **G. and** Isenberg, **D. (2014):** Systemic lupus erythematosus. The Lancet, 384(9957). DOI: 10.1016/S0140-6736(14)60128-8.

Martinello, M. and Mutinelli, F. (2021): Antioxidant activity in bee products. Antioxidants, 10: 71-108. https://doi.org/10.3390/antiox10010071.

Mohammed, Z. and Hassan, A. (2019): Effect of bee venom on some blood and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug. IOP Conference Series: Journal of Physics: Conference Series, 1234: 012066. Doi:10.1088/1742-6596/1234/1/012066.

Muhammad, M.; Mouchira, M. and Naglaa, R. (2015): Physiological effects of bee venom and propolis on on irradiated Albino rats. Danish Journal of Agriculture Animal Science,11-21.

Ozdemir, C.; Kucuksezer, U.; Akdis, M. and Akdis, C. (2011): Mechanisms of immunotherapy to wasp and bee venom. Clin. Exp. Allergy, 41:1226–1234.

Rabie, A. H.; El-Kaiaty, A. M.; Hassan, M.S. and Stino, F. K. (2018): Influence of some honey bee products and a growth promoter supplementation on productive and physiological performance of broiler chickens. Egyptian Poultry Science Journal, 38:513–531.

Schumacher, M. J.; Schmidt J. O. and Egen, W. B. (1989):

Lethality of "killer" bee stings. Nature, 337: 413.

Seo, D. M.; Park; D. S. and Kang, S. G. (2003): The analgesic effect of bee venom aqua acupuncture and its mechanism in the rat model with adjuvant induced arthritis. Journal of Korean Acupuncture and Moxibustion Medicine Society, 20:85–97.

Shamim, R.; Farman, S.; Batool, S.; Khan, S. E. A. and Raja, M. K. H. (2022): Association of systemic lupus erythematosus disease activity index score with clinical and laboratory parameters in pediatric onset systemic lupus erythematosus. Pakistan Journal of Medical Science, 36 (3): 467- 472.

Sharma, P.; **Joshi, R. V.; Pritchard, R.; Xu, K. and Eicher, M. A. (2023):** Therapeutic Antibodies in Medicine. Molecules, 28(18): 6438; https://doi.org/10.3390/molecules28186438

Son, D. J.; Lee, J. W.; Lee, Y. H., *et al.* (2007): Therapeutic application of anti-arthritis, painreleasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacology and therapeutics, 115(2):246-270.

Song, G. G.; Rho, Y. H.; Woo, J. H.; Choi, S. J.; Lee, Y. H.; Ji, J. D. and Yu, W. (2009): A New onset of systemic lupus erythematosus developed after bee venom therapy. Korean Journal of International Medicine, 24: 283–285.

Sturm, G.; Kranke, B.; Rudolph, C. and Aberer, W. (2002): Rush hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. Journal of Allergy and Clinical Immunology, 110: 928–933. https://doi.org/10.1067/mai.2002.129124.

Varanda, E. A.; Monti,R. and Tavares, D. C. (1999): Inhibitory effect of propolis and bee venom on the mutagenicity of some direct and indirect-acting mutagens. Teratogens, Carcinogens and Mutagens Reproductive Toxins, 19: 403:413.

Yapici, I.; Isol, E. and Gulcin, I. (2023): The effects of bee venom in the treatment of diseases. In book: The significance of bee products in health (PP. 9-17). Orient Publication. https://www.researchgate.net/publication/370708626.

Yousefpoor, Y.; Osanloo, M.; Mirzaei-Parsa, M. J.; Hoseini, M. R. M. R.; Hashemi, N. S. M. and Abbasifard, M. (2022): Subcutaneous injection of bee venom in Wistar rats: Effects on blood cells and biochemical parameters. Journal of Pharmacopuncture, 25(3):250-257. https://doi.org/10.3831/KPI.2022.25.3.250.