BENHA VETERINARY MEDICAL JOURNAL, VOL. 25, NO. 1:183-191, SEPTEMBER 2013

STUDY OF THE IMMUNOLOGICAL EFFECT OF BEE VENOM ON CHRONIC DISEASES IN HUMAN

Sabah F. El-Abd[1], Abir A. Elfiky[2], Essam-Eldin A. Mashhoor[2],
[1]Genetic engineering and biotechnology institute. [2]ANDI center of excellence in anti-venom research, EGYVAC, VACSERA.

A B S T R A C T

This study was performed on thirty (30) patients who were received bee venom therapy intradermaly in a dose of 300 µg/ml twice weekly for 12 months, preceded by rush immunotherapy schedule for one week. All patients received bee venom therapy in VACSERA. . Evaluation of our patients during the follow up period was based on hematological, biochemical, and immunological investigations. to evaluate the effectiveness of bee venom in treating chronic Hepatitis C virus (HCV) results revealed a highly significant increase in the mean platelets, white blood cells (WBCs) and lymphocytes level, while there was no significant changes in the Hb or RBC or the granulocytes count. there was significant decrease in liver enzyme including alanine transferase (ALT) and Aspartate amino transferase (AST) Immunological investigation of HCV, Immunoglobulin-G (IgG), and Immunoglobulin-E (IgE) was applied on all patients Pre- treatment then at 6 months and 12 months post-treatment with bee venom . revealed a highly significant decrease in the IgE level and a highly significant increase in the IgG level after 6 month and 12 month of Treatment Determination of HCV RNA by RT-PCR showed But of 30 patients 11 patients (37 %) were negative and markedly decreased in 16 patients (53 %), while 3 patients (10 %) show no decrease in the RT- PCR results after12 month of bee venom injection.

Keywords: Bee venom, Hepatitis C virus (HCV), QRT-PCR (Quantitative Reverse Transcriptase PCR).

(BVMJ-25(1): 183-191, 2013)

1. INTRODUCTION

Over 170 million individuals are infected with hepatitis-C virus (HCV) worldwide [1]. Hepatitis C prevalence is higher in some countries in Africa and Asia. The report published by the Egyptian Demographic Health Survey (EDHS) stated that, in Egypt, there is an overall Anti-HCV antibody prevalence of 14.7% (estimated to be about 12 million individuals) and the number of Egyptians estimated to be chronically infected was 9.8% (about 8 million individuals) [2]. Hepatitis C virus represents the second most common blood-borne illness in the world, affecting up to 2% of the world's population. About 110-300 One hundred to 300 million people worldwide are estimated to be infected with HCV. Seventy-five percent will develop chronic infection and at least 15–20% of these individuals will eventually develop cirrhosis and are at risk of developing complications of end stage liver disease [3]. Data suggest that HCV infection should be regarded as a systemic infection with multi-organ involvement (HCV can affect renal, hematological, pulmonary and immune systems) [4] . Bee venom therapy (BVT) is the application of live honeybee stings to patients for therapeutic purposes. BVT is one of the most frequently used

traditional complementary and alternative therapeutic methods and has long been believed to be effective in the treatment of many diseases, including rheumatic arthritis, bursitis, tendinitis, shingles (herpes zoster), multiple sclerosis, wounds, gout, burns and infections [5] , [6]. Further studies are needed to evaluate effect of bee venom components on chronic hepatitis C. In this paper we investigate the effect of bee venom on different parameters' of patients with chronic HCV infection.

1. MATERIAL AND METHODS

1.1. Patients

30 Patients with chronic hepatitis-C (HCV) were selected for this study (after written consent) the current study was conducted at the holding company for biological products and vaccines (VACSERA). All investigations were done at vacsera labs. All patients were subjected to the following before the study, Detailed medical history, Clinical examination, Laboratory investigation, Inclusion Criteria: 1) Chronic hepatitis-C infection proved by positive HCV antibody and positive serum HCV-RNA detected by polymerase chain reaction (PCR) (Quantitative). 2) Elevated serum alanine amino transferase two folds or more above the normal levels for 12 months. Exclusion Criteria: Patients with liver cell failure, Patients with renal failure, Patients with heart failure, and Patients with hemophilia.

1.2. Bee venom.

Bee venom used at this study was prepared by vacsera under the trade name of ABEVAC (purified bee venom from Apis mellifera species at concentration 1:1, and kept in special vial containing 1ml in each). The studied cases were all treated with bee venom by intradermal injection for 12 months according to the schedule of injection. Follow up and laboratory investigation was applied on patients every 12 weeks.

Dosages:

VACSERA suggested a schedule for chronic HCV patients. This special injection was taken from [7]. Protocol of Rush Immunotherapy. This schedule is in conformance with the scientific literature recommendations which have been published in this field [8] Clinical studies on the use of animal toxins (bee venom) as an alternative therapy in the veterinary practice [9].Safety study of bee venom preparation. Scientifical annual meeting, Scientifical annual meeting, National Organization for Drug Control and Research [10].

1.3. Samples.

- Blood samples:

From each patient (every 3 months till the end of the experiment) about 2 ml whole blood were taken in vacutainers, containing EDTA, or Citrate as an anticoagulant. These samples were used for determining the hematological picture.

- Serum samples:

Blood samples from each patient were taken simultaneously as before in vacutainers but without addition of any anticoagulant. The obtained serums were used for determination of different blood chemistry, and immunological parameters.

1.4. Hematological analysis

The hematological values: Red blood cells (RBCs) count, Hemoglobin content, White blood cells (WBCs) count, and platelets count was determined using coulter counter.

1.5. Liver function test

Determination of serum activity level of Alanine amino transferase (ALT) was carried out according to International Federation of Clinical Chemistry and Determination of serum activity level of aspartate amino transferase (AST) was

carried out according to International Federation of Clinical Chemistry [11].

1.6. Detection of HCV IgG and IgE (Immunological Investigation)

Determination of serum immunoglobulins (IgG) level:-
Determination of IgG serum levels according to [12]. Determination of serum immunoglobulins (IgE) level:- Determination of serum IgE level according to [13].

1.7. Detection of HCV RNA using Quantitative RT – PCR.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR): The protocol for performing RT-PCR to detect HCV RNA was performed according to[14].with modifications to increase the sensitivity of the assay. Sensitivity of the assay is 50 IU/ml.

1.8. Statistical analysis.

The results are represented as mean ± S.E. Results were analyzed statistically by SPSS (Statistical program for social science [15].

2. RESULTS

2.1. Safety results

During all the study we found that there were no systemic side effects nor allergy from bee venom reported while there were only local allergy at the site of injection which disappeared after 48 hours of the injection as a maximum time during all the follow – up period.

2.2. Hematological examination

Regarding the hematological examination (table 1), RBC, and Hb level showed that treatment of patients with bee venom resulted in non significant changes in both along the test period of bee venom injection ($p>0.05$). The Platelet count and WBCs count showed that treatment of patients with bee venom resulted in a highly significant increase within the normal range in the mean platelet level ($p<0.01$) after 3 months, 6 month, 9 month, and after 12 months of bee venom injection. The differential leukocytes count level (Table 2) showed that treatment of patients with bee venom resulted in a highly significant increase in the mean lymphocytes level (within the normal range) ($p<0.01$) along the test period, While showed that treatment of patients with bee venom resulted in non-significant changes along the test period as regard monocytes, neutrophils, basophils and eosinophils during follow up period by paired t-test ($p>0.05$).

2.3. Liver activities

Regarding biochemical investigation, ALT and AST activity levels (Table 3) showed that treatment of patients with bee venom resulted in a highly significant decrease ($P\leq0.00$) in ALT level starting from the 3 rd month, and along the test period of bee venom injection, while AST activity level resulted in significant decrease in AST level ($P\leq0.05$) after 3 months, and a highly significant decrease in AST level ($P\leq0.00$) after 6 month, 9 month, and after 12 months of bee venom injection.

Table 1: Schedule of bee venom administration for chronic HCV patients

days of administration	dose / patient
1st	50µg
3rd	110 µg
5th	200µg
7th	300 µg
Maintenance dose (every other day)	300 µg

2.4. Immunological examination

Regarding the immunological investigation (Table 4) showed that treatment of patients with bee venom resulted in a highly significant increase in the IgG level after 6 month and 12 month ($p<0.01$) by bee venom

injection, while IgE level showed highly significant decrease in the IgE level after 6 month and 12 month (p<0.001) by bee venom injection, The PCR level showed a highly significant decrease in its level after 6 month and 12 month (p<0.001) by bee venom injection.

Table 2: Results of hematological examination before and after bee venom therapy.

Follow – up period	RBCs ($*11^6/mm^3$)	Hb (g/dl)	Platelets ($*11^3/mm^3$)	WBCs ($*11^3/mm^3$)
Before treatment	0.56.1+	14.2±1.3	186.6±37.9	6.0±2.04
3rd month	6.4 ±0.5	14.3±1.03	191.3^{***}±480	6.5^{**}±1.8
6th month	6.2±0.3	14.4±0.8	193.8^{**}±37.9	6.8^{***}±3.03
9th month	6.0±0.4	14.4±1.01	209.3^{***}±35.9	6.5^{*}±2.2
12thmonth	6.3±o.5	14.5±0.9	211.2^{***}±35.6	6.9^{***}±2.1

The results are represented as mean ± S.E. *P≤0.05, **P≤0.01, ***P≤0.001

Table 3: Result of Mean Differential leukocytes count level before and after bee venom therapy.

Follow – up period	Lymphocytes	Monocytes	Eosinophil	Basophile	Neutrophil
Before treatment	2.9±0.7	4.1±0.1	0.07±0.02	0.005±0.005	3.6±0.2
3rd month	3.3^{**}±0.6	3.8±0.4	0.07±0.01	0.004±0.006	3.5±0.5
6th month	3.5^{***}±0.8	3.7±0.1	0.08±0.01	0.006±0.004	3.6±0.3
9th month	3.6^{***}±0.7	3.6±0.9	0.08±0.01	0.003±0.006	3.6±0.4
12thmonth	4.0^{***}±0.4	3.6±0.1	0.09±0.02	0.002±0.006	3.7±0.5

Table 4: Results of biochemical examination for liver enzymes before and after bee venom therapy

Follow – up period	ALT (IU/L)	AST (IU/L)
Before treatment	112.4±39.5	69.2± 24.7
3th month	65.1^{***}± 28.4	58.3^{*}±20.0
6th month	62.6^{***}± 23.5	53.26^{***}±18.3
9thmonth	65.5^{***}±26.9	52.6^{***}±15.0
12th month	57.3^{***}±14.3	42.2^{***}±12.7

Table 5: Results of immunological investigation of HCV IgG and IgE beside HCV RNA detection by QRT-PCR pre& post Treatment with bee venom therapy.

Follow – up period	IgG (mg/dl)	IgE (IU/ml)	PCR ($*11^6$ copies/ml)
Before treatment	1502.3±361.3	31.5±11.1	6.6±4.4
6th month	1601.2^{***}±3932	26.2^{***}±11.5	2.4^{***}±2.1
12thmonth	1675.1^{***}±172.8	23.2^{***}±9.2	1.4^{***}±1.0

3. DISCUSSION

Statistical analysis of the results revealed that there were no significant changes in the RBCs, and Hb level along the test period. while there was a highly significant increase in the mean WBC level ($p < 0.01$) within the normal range concomitant with a highly significant increase in the mean lymphocytic count (within the normal range) ($p < 0.01$) along the test period of bee venom injection. This may be due to enhancement of lymphocytosis under the effect of bee venom components as phospholipase (PLA2) which discussed by [16], or due to lymphocytes proliferation by stimulation of immune system because of bee venom injection, [17]. Concerning granulocytes (neutrophils, eosinophils, and basophile count) there were no significant changes along the test period ($p>0.05$). These results were in agreement with [18] and may be due to the injection of non-toxic dose as mentioned by [19]. By follow up, the platelets count in our study there was a highly significant increase in the mean platelet level (within the normal range) ($p < 0.01$) during follow up period of bee venom injection. This platelets activity may be due to bee venom hypersensitivity [20] but the use of bee venom in special schedule for injection may cause desensitization which give rise to adjustment of platelets level to be in the normal range. Also may be due to inhibition in platelets aggregation under the stimulatory effect for production of prostaglandin as mentioned by [21]. In contrast [22] concluded that phospholipases A2 induce platelet aggregation and may be responsible for anti-platelet activity. Regarding biochemical results there was a highly significant decrease in ALT, AST ($P\leq0.01$) starting from the 3rd month, and along the test period of bee venom injection. As the decrease in these enzymes activity can be considered as indicator for bee venom anti-inflammatory effect or its activity for regeneration .the present results also agree with the results of [23] who used melittin to construct recombinant adenovirus in an attempt to achieve a specific killing action on liver cancer and improve hepatic function in cirrhotic livers infected with hepatitis virus. Regarding immunological evaluation of bee venom injection we found that serum IgG level showed a highly significant increase after 6 months and at the end of the study (12 month) ($P<0.01$). This result may be attributed to melittin structure which has the ability to bind the cell membrane giving rise to immunogenecity for IgG response as previously mentioned by [24] or may be due to rapid shift in cytokine expression Th2 to Th1 and induction of IL11 at first by T cells and later by B cells and monocytes by the effect of bee venom immunotherapy. It is well known that IL1 promote production of IgG and T cell immunity as reported by ([25, 26, and 27]. Concerning Serum IgE level showed significant decrease after 6 months and at the end of the study (12 month) ($P<0.01$). This finding was in agreement with [28], of [29], and may be attributed to the following: firstly rapid shift in cytokine expression Th2 to Th1 and induction of IL11 by BVT or due to bee venom phospholipase A2 which eliminates a population of early activated CD8-Tcell that regulate IgE population, and also due to unchanged in CD28 expression pathway which probably involved in allergic reaction at least at the phenotypic level after rush venom immunotherapy. From the previously mentioned data, we could explain that this decrease in IgE means decrease in systemic allergic reaction as previously reported by [30]. Regarding to serum HCV- RNA detected by Quantitative RT- PCR, there was significant decrease along the test period ($P<0.01$). At the end of the study we found that 11 patients had a negative percent result (37%), 16 patients showed marked decrease in their serum HCV- RNA by PCR (53 % of cases). While three patients show no decrease in the PCR result after 12 month of bee venom injection (11%). This percentage of HCV - RNA negative and

that of decrease are highly promising when compared with interferon - ribavirin combination therapy which didn't exceed 20.8 % [31, 32]. Also the results of bee venom therapy for chronic HCV infection are encouraging when compared with interferon - amantadine, interferon - ribavirin – amantadine combination modalities tried before [33, 34, 35]. During all the study we found that there were no systemic side effects nor allergy from bee venom reported while there were only local allergy at the site of injection which disappeared after 48 hours of the injection as a maximum time during all the follow – up period.

Conclusion

It could be concluded that bee venom injection in aqueous preparations is a safe medication for treatment of chronic HCV infection. The use of bee venom therapy is useful to treat HCV patients by maintain their health condition and immune status of the patients. No systemic side effects or allergy reported during the follow up period and all patients continued the treatment for 12 months.

4. REFERENCES:

1. Baldo, V.; Baldovin, T.; Trivello, R, (2008): Epidemiology of HCV infection: Curr Pharm Des; 14:1646-1654.
2. Dewolfe, M. and AbuRaddad, L. (2011): Proceedings of the National Academy of Sciences of the United States of America [PNAS].
3. Meguid M.A. and Moussa M. (2011). Cognitive Function in Hepatitis C Patients: Effect of Pegylated Interferon α and Ribavirin Therapy. Current Psychiatry; 17(2): 45-51.
4. Puchner, K.P. and Berg, T. (2009): Hepatology and Gastroenterology Clinic, University of Berlin: May; 47(5):446-456.
5. Chen, J.; and Lariviere, W.R. (2011):The nociceptive and anti-nociceptive effects of bee venom injection and therapy: a double-edged sword. Prog Neurobiol;92: 151–83.
6. Choi M S , Park S, Choi T, Lee G, Haam K-K, Hong M-C, Min B-I, Bae H(2013). Bee venom ameliorates ovalbumin induced allergic asthma via modulating CD4+CD25+ regulatory T cells in mice. Cytokine 61: 256–265.
7. Diez-Gomez, M.L.; Quirce-Gancedo,S. and Julia –de-paramo,B. (1995) :Venom immunotherapy : tolerance to a 3-day protocol of ruch- immunotherapy. Allergol. Immunopathol - (Madr) 1995 Nov – DEC.; 23 (6) 277 – 84.
8. Bernstein, J.A.; Kagen, S .L; Bernstein, D .I. and Bernstein, I . L. (1994) :Rapid venom immunotherapy is safe for routine use in the treatment of patients with hymenoptera anaphylaxis Ann – Allergy; Nov., 73 (5) : 423
9. Abir A. H. Elfiky (2006): Clinical studies on the use of animal toxins (bee venom) as an alternative therapy in the veterinary practice. (Ph-D) published seminar at Faculty of Veterinary Medicine Cairo University, November 2006.
10. Abir A. Elfiky, Elsherif M. T., Elhamamsy H. T., Ramadan S. A. (2007): Safety study of bee venom preparation. Scientifical annual meeting, Scientifical annual meeting, National Organization for Drug Control and Research, Jan 2007. (Poster)
11. International Federation of clinical chemistry (1980): Scientific committee. J. Clin. Chem. Clin. Biochem.18:521-534.
12. Thomas, L. (1995): Neue Normal bereiche fur 14 Serum proteine. Deatches Arzteblatt 1995:36 A: 2337. B: 1742, C: 1563.
13. World Health Organization (1994): Expert committee on biological

standardization. WHO Tech Rep Ser 840: 7.
14. Abdel-Hamid M., Edelman D.C. and Highsmith W.E. (1997): Optimization, assessment, and proposed use of Direct Nested Reverse Transcription-Polymerase Chain Reaction protocol for the detection of hepatitis C. J of Human Virol. 1: 58-65.
15. SPSS (1998): Statistical Package for Social Science. Computer Software, Ver. 11. SPSS Company, London, UK.
16. Prinz J.C.; Endres N.; Rank G.; Ring J.; and Rieber E.P. (1987): Expression of Fc epsilon receptors on activated human T lymphocytes .Eur .J.Immunol 17(6):757-61.
17. Lomnitzer R.; and Rabson A.R. (1986): Lack of responsiveness of beekeeper mononuclear cells to in vitro stimulation with pure bee venom .J Allergy Clin Immunol.; 78 (1): 25-30.
18. Somerfield, S.D.; Stach, J.L.; M raz, C.; Gervais, F.; and Skamene, E.(1984) : Bee venom inhibits superoxide production by human neutrophils . Inflammation. Dec.; 8 (4):385–91.
19. Kosnik M.,and Wraber B.(2000):Shift from Th2 to Th1 response in immunotherapy with venoms. Pflugers Arch. 440 (5 Suppl):R70-1.
20. Tsicopoulos A.; Tonnel A. B.; Wallaert B.; Joseph M.; Ramon P.; Capron A. (1989): A circulating suppressive factor of platelet cytotoxic functions after rush immunotherapy in Hymenoptera venom hypersensitivity. J. Immunol. 142(8): 2683-8.
21. Ribardo D.A.; Crowe S.E.; Kuhl K.R.; Peterson J.W.; and Chopra A.K. (2001): Prostaglandin levels in stimulated macrophages are controlled by phospholipase A2 –activating protein and by activation of phospholipase C and D. J. Biol. Chem. 276 (8): 5467-75.
22. Ouyang C and, Huang TF. Effect of the purified phospholipases A2 from snake and bee venoms on rabbit platelet function. Toxicon. 22 (5):705-18.
23. Ling, C.Q., Li, B., Zhang, C., Zhu, D.Z., Huang, X.Q., Gu, W. (2005). Inhibitory effect of recombinant adenovirus carrying melittin gene on hepatocellular carcinoma. Ann Oncol. 16(1): 119-115.
24. Fehlner, P.F.; Kochoumian, L.; and King, T.P. (1991): Murine IgE and IgG4 responses to melittin and its analogs. J. Immunol. 146 (8): 2664-70.
25. Bellinghausen, I., Metz, G., Enk, A. H., Christmann, S ., Knop ,J. and Saloga, J. (1997):Venom immunotherapy induces interleukin –11 production and Th2 to Th1 shift , and changes surface marker expression in venom – allergic subjects . Eur J. Immunol; 27 (5):1131 -9.
26. Akdis, C.A., Blesken, T., Akdis, M., Wuthrich, B .and Blaser, K. (1998): Role of interleukin 11 in specific immunotherapy. J. Clin. Invest. 112(1): 98-116.
27. Akdis, C.A. and Blaser, K. (1999): IL -11 –induced energy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy .F A S E B J. 13 (6): 603-9.
28. Diaz-Sanchez D.; Noble A.; Staynov D.Z.; Lee. T.H. and Kemeny D.M. (1993): Elimination of IgE regulatory rat CD8+T cells in vivo differentially modulates interleukin -4 and interferon-gamma but not interleukin -2 production by splenic T cells Immunology.; 78(4): 513-9.
29. Tsicopoulos A.; Labalette M.; Akoum H.; Dues C.; Wallaert B.; Dessaint J. P.; and Tonnel A. B. (1996): CD28 expression is increased in venom allergic patients but is not modified by specific immunotherapy. Clin. Exp. Allergy. 26 (11): 1119-24.
30. Muller, U. R.; Morris, T.; Bischof, M.; Friedi, H. and Skarvil, F. (1986): Combined active and passive

immunotherapy in honeybee sting allergy. Journal of Allergy and Clinical immunology, Pages 78(1): 115-122.

31. EL- Zayadi, A.; Selim, O.; Simmonds, P.; Bahget, M.; Dabbous, H.; Zakaria, S.; Montasser, M . F .and Ahmed, A. (1997): Serotyping of HCV in Egyptian chronic hepatitis C virus patients. The Egypt J of Internal Medicine; 6 (2):545–52.
32. EL–Zayadi, A.; Selim, O.; Hadad, S.; Simmonds, p.; Hamdy, H.; Badran, H.M. and Shawky, S. (1999): Combination treatment of interferon alpha-2b and ribavirin in comparison to interferon monotherapy in treatment of chronic HCV genotype 4 patients Ital , J .Gastroenterol Hepatoi 31.
33. Smith, J.P. (1997): Treatment of chronic hepatitis C with a mantadine .Digestive Dis Sci; 42(8):1681-7.
34. Mc Hutchison, J. and Younossi, Z .M. (1999): The roles of amantadine, riemantadine, ursodexycholic acid and NSAIDs, alone or in combination with alpha interferon, in treatment of chronic hepatitis C. Semin– Liver Dis; 19 (Suppl. 1): 57– 65.
35. Brillanti, S. M.; Foli, Di Tamaso,L; Masci, C. and Bolon, L. (1999) : Pilot study of triple antiviral therapy for chronic hepatitis C in interferon alpha non responders .Ital J Gastroenterol Hepatol; 31:131-4.

دراسة التأثير المناعي لسم النحل على الامراض المزمنة فى الانسان

الملخص العربي

العلاج بسم النحل بدا فعليا وعلى اساس علمي منذ ما يزيد عن 60عاما وانتشر الآن فى عدد من الدول. كما نجد ان العلاج بسم النحل يستخدم الان على نطاق واسع فى علاج كثير من الامراض مثل الالتهابات الفيروسية والالتهابات البكتيرية والامراض المناعية والامراض السرطانية وكثير من الامراض الاخرى. ويعتبر العلاج بسم النحل جزء من العلاج بمنتجات النحل التي تعتمد بدورها على منتجات الخلايا المختلفة والتي يمكن استخدامها فى العلاج لحالات الامراض المزمنة. ويحتوي سم النحل على ببتيدات مهمة وهي كالاتي الميليتين والابامين بحيث يقوم كل منهما بأثارة الغدة النخامية والكظرية للإفراز الكوريتزون بينما يعتبر الببتيد 401 مضاد للالتهابات. ويعتبر سم النحل من المواد ذات التأثير المضاد للفيروسات من خلال مكوناته المتمثلة فى الفوسفوليب يز 2 أ والبروتياز أنهيبيتورز. وقد تم عمل بعض الدراسات الخاصة بذلك بالمصل واللقاح (فاكسيرا). وهناك العديد من البروتوكولات للعلاج لتجنب حدوث أي أعراض جانبية. وقد اجريت الدراسة الحالية على عدد ثلاثون مريضا بالالتهاب الكبدي سى وتم اعطائهم جرعات تدريجية وتلتها جرعة 300 ميكروجرام يوم بعد يوم لمدة 12 شهر فى الجلد. وقد أظهرت هذه الدراسة ان العلاج بسم النحل لمرضى الا لتهاب الفيروسي (سى) ادى الى انخفاض انزيمات الكبد اثناء وبعد 12 شهر من بداية العلاج. كما انخفضت نسبة الفيروس فى الدم ،حيث ان العلاج بسم النحل ادى الى اختفائه تماما فى 11 مريض (37%) وقلت النسبة فى 16 مريض (53%) و 3 مرضى(11%) لم يحدث معهم تغير فى نسبة الفيروس فى الدم. فى خلال عام من العلاج بسم النحل لم يظهر له أي اعراض جانبية عامه ولكن نلاحظ وجود احمرار فى مكان الحقن وينتهي فيما لا يزيد عن 48 ساعة من الحقن.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(1):183–191, سبتمبر 2013)