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

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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 \textmu g/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).

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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. Scientific annual meeting, Scientific 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≤0.00) 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≤0.05) after 3 months, and a highly significant decrease in AST level (P≤0.00) after 6 month, 9 month, and after 12 months of bee venom injection.

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

<table>
<thead>
<tr>
<th>days of administration</th>
<th>dose / patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>50μg</td>
</tr>
<tr>
<td>3rd</td>
<td>110 μg</td>
</tr>
<tr>
<td>5th</td>
<td>200μg</td>
</tr>
<tr>
<td>7th</td>
<td>300 μg</td>
</tr>
<tr>
<td>Maintenance dose</td>
<td>300 μg</td>
</tr>
<tr>
<td>(every other day)</td>
<td></td>
</tr>
</tbody>
</table>

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
Study of the immunological effect of bee venom on chronic diseases in human

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 high decrease after 6 month and 12 month ($p<0.001$) by bee venom injection.

### 3. DISCUSSION

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

<table>
<thead>
<tr>
<th>Follow-up period</th>
<th>RBCs (*10^6/mm³)</th>
<th>Hb (g/dl)</th>
<th>Platelets (*10^11/mm³)</th>
<th>WBCs (*10^11/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>5.56±0.1</td>
<td>14.2±1.3</td>
<td>186.6±3.79</td>
<td>6.0±0.24</td>
</tr>
<tr>
<td>3rd month</td>
<td>6.4±0.5</td>
<td>14.3±1.03</td>
<td>191.3***±480</td>
<td>6.5***±1.8</td>
</tr>
<tr>
<td>6th month</td>
<td>6.2±0.3</td>
<td>14.4±0.8</td>
<td>193.8**±37.9</td>
<td>6.8***±3.03</td>
</tr>
<tr>
<td>9th month</td>
<td>6.0±0.4</td>
<td>14.4±1.01</td>
<td>209.3***±35.9</td>
<td>6.5±2.2</td>
</tr>
<tr>
<td>12th month</td>
<td>6.3±0.5</td>
<td>14.5±0.9</td>
<td>211.2***±35.6</td>
<td>6.9***±2.1</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Follow-up period</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophil</th>
<th>Basophile</th>
<th>Neutrophil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>2.9±0.7</td>
<td>4.1±0.1</td>
<td>0.07±0.02</td>
<td>0.005±0.005</td>
<td>3.6±0.2</td>
</tr>
<tr>
<td>3rd month</td>
<td>3.3***±0.6</td>
<td>3.8±0.4</td>
<td>0.07±0.01</td>
<td>0.004±0.006</td>
<td>3.5±0.5</td>
</tr>
<tr>
<td>6th month</td>
<td>3.5***±0.8</td>
<td>3.7±0.1</td>
<td>0.08±0.01</td>
<td>0.006±0.004</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td>9th month</td>
<td>3.6***±0.7</td>
<td>3.6±0.9</td>
<td>0.08±0.01</td>
<td>0.003±0.006</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>12th month</td>
<td>4.0***±0.4</td>
<td>3.6±0.1</td>
<td>0.09±0.02</td>
<td>0.002±0.006</td>
<td>3.7±0.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Follow-up period</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>112.4±39.5</td>
<td>69.2±24.7</td>
</tr>
<tr>
<td>3rd month</td>
<td>65.1***±28.4</td>
<td>58.3*±20.0</td>
</tr>
<tr>
<td>6th month</td>
<td>62.6***±23.5</td>
<td>53.26***±18.3</td>
</tr>
<tr>
<td>9th month</td>
<td>65.5***±26.9</td>
<td>52.6***±15.0</td>
</tr>
<tr>
<td>12th month</td>
<td>57.3***±14.3</td>
<td>42.2***±12.7</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Follow-up period</th>
<th>IgG (mg/dl)</th>
<th>IgE (IU/ml)</th>
<th>PCR (*10^6 copies/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>1502.3±361.3</td>
<td>31.5±11.1</td>
<td>6.6±4.4</td>
</tr>
<tr>
<td>6th month</td>
<td>1601.2***±3932</td>
<td>26.2***±11.5</td>
<td>2.4***±2.1</td>
</tr>
<tr>
<td>12th month</td>
<td>1675.1***±172.8</td>
<td>23.2***±9.2</td>
<td>1.4***±1.0</td>
</tr>
</tbody>
</table>

highly significant decrease in its level after 6 month and 12 month ($p<0.001$) by bee venom injection.
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 ($P \leq 0.01$) 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 immunogenicity 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:**


Study of the immunological effect of bee venom on chronic diseases in human

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دراسة التأثير المناعي لسم النحل على الأمراض المزمنة في الإنسان

الملخص العربي

العلاج بسم النحل بدأ فعليا وعلى اساس علمي منذ ما يزيد عن 60 عاما وانشتر الآن في عدد من الدول. كما نجد أن العلاج بسم النحل يستخدم الآن على نطاق واسع في علاج كثير من الأمراض مثل الالتهابات الفيروسية والالتهابات البكتيرية والأمراض المناعية والأمراض السرطانية وكثر من الأمراض الأخرى. ويعتبر العلاج بسم النحل جزء من العلاج بعشرات النحل التي تعتمد بدورها على منتجات الخلايا المختلفة والتي يمكن استخدامها في العلاج لحالات الأمراض المزمنة. ويتحبس سم النحل على ببتيدات متحركة وهي كالأتي الميلينين والألبامين حيث يقوم كل منها بإثارة الغدد الداخلية والكطرية لإفراز الكورتيزون بينما يعتبر الببتيد 401 مضاد للالتهابات. ويعتبر سم النحل من المواد ذات التأثير المضاد للفيروسات من خلال مكوناته المتمثلة في الفوسفويليبز 2 أ والبروتياز أندبيتورز. وقد تم عمل بعض الدراسات الخاصة بذلك بالعمل واللقاح (فاكسيرا). وهناك العديد من البروتوكولات للعلاج لتجنب حدوث أي أعراض جانبية. وقد أجريت الدراسة الحالية على عدد ثلاثون مريضا بالالتهاب الكبدية ومن اعطائهم جرعات تدريجية وثقتها جرعة 300 ميكروجرام يوم بعد يوم لمدة 12 شهرا في الجلد. وقد أظهرت هذه الدراسة ان العلاج بسم النحل لم يسبب أي ردود فعل في 37٪ من المرضى (37٪) واجهت أنه تم التحسن بعد 12 شهر من بداية العلاج. كما انخفضت نسبة الفيروس في الدم حيث ان العلاج بسم النحل أدى إلى اخفاقه تماما في 11 مريض (37٪) وقلت النسبة في 16 مريض (53٪) و3 مرضى (11٪) لم يحدث معهم تغيير في نسبة الفيروس في الدم. في خلال عام من العلاج بسم النحل لم يظهر له أي أعراض جانبيه عامه ولكن نلاحظ وجود احمرار في مكان الحقن ويتنهي فيما لا يزيد عن 48 ساعة من الحقن.