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Natural Honey Bee venom Manipulates Human Immune Response

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ABSTRACT

Background: Honey bee venom is an important toxin which has various useful properties. Bee venom possesses various peptides including melittin, apamin, adolapamin and mast cell degranulating peptide. It appears to stimulate cortisone secretion, enhances antibody production, and affects cytokine production. **Aim:** The aim of the work is to study changes in levels of complement system proteins, C3 and C4, together with C-reactive proteins and rheumatoid factors (CRP and RF) in response to bee venom in subjects exposed naturally to sting with honey bee workers. **Subjects and Methods:** Subjects (12) were randomly selected from patients visiting El-Mostafa bee house, Elmarg, cairo, Egypt; to get natural bee venom therapy for various diseases. Blood samples (24) were collected from these volunteers, before and after honey bee sting, at the laboratory of Egypt Air Hospital, Cairo, Egypt following the standard protocol. Serum levels of C3 and C4 were measured by radial immunodiffusion technique. CRP was quantitatively measured by in vitro diagnostic test using auto analyzer (Dimension® EXEL) clinical chemistry system. Serum RF was measured by Rapid latex agglutination test for qualitative screening. **Result:** After exposure of patients to bee sting: all abnormal levels of C3 returned to normal values while abnormal C4 levels did not change; Half of cases that were showing abnormal high CRP levels have dropped to normal levels and All RF positive cases have become negative. **Conclusion:** The complement system has been activated, in patients, by both classical and alternative pathways before treatment with bee venom where it became only classically activated after treatment. Improved values of both CRP and RF indicate reduction in the inflammatory immune response after exposure of patients to honey bee venom.

Key words: Honey bee, Venom, Human, C3, C4, CRP, RF, Egypt

INTRODUCTION

Apitherapy is nowadays practiced all over the world. Use of honey and other bee products can also be traced back thousands of years. Apitherapy has been practiced in: ancient Egypt, Greece and China for as old as 3-5000 years ago[(1)]. Holy Quran has paid our attention to the importance of bees in life more than 1400 years ago by including a separate complete chapter 16, Surat An-Nahl, entitled by the name of bees where you find two verses; 68 and 69 talking about the life of bees and healing properties of its secretions.

Bee venom is a natural toxin produced by the honey bee and it has a prime role of defense for the bee colony. It has an efficient and complex mixture of substances designed to protect bees against a broad diversity of predators. Bee venom possesses various peptides including melittin, apamin, adolapamin and mast cell degranulating peptide. It also contains enzymes, biologically activity amines and non-peptide components. Enzymes are composed of phospholipase A2 (PLA2), hyaluronidase, acid phosphomonesterase, α-D-glucosidase and lysophospholipase, as well as non-peptides such as histamine, dopamine and norepinephrine [(2)].

The role of the complement as a system merging early-phase innate immunity with later-phase acquired immunity has been established. Complement protein 3 (C3) is a key protein of the complement system. Activation of C3 results in a variety of immunologic reactions such as immune adherence, phagocytosis, antibody response, cytolysis, inflammation, and killing of pathogenic microorganisms[(3,4)].

Complement protein 4 (C4) plays a central role in classical and lectin pathways of complement. A C4 deficiency is often seen in association with infectious diseases [(5,6)].

C-reactive protein (CRP) is a well-known inflammatory marker which is able to activate complement component [(7)].

Human studies have shown bee venom to be immensely beneficial in rheumatoid arthritis (RA, positive RF auto antibodies) patients and possess anti-inflammatory and antioxidant activity [(8)]. Bee venom has also been used in oriental medicine to relieve pain and to treat chronic inflammatory diseases [(9)]. Bee venom therapy has also been used as the therapeutic method in treating rheumatoid arthritis, amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, liver fibrosis, atherosclerosis, pain and others[(10)].

The aim of the present work is to Study changes in levels of C3 and C4 together with CRP and RF in response to bee venom in patients exposed naturally to sting by honey bee workers.

PATIENTS AND METHODS

Patients: Patients (12) were randomly selected from subjects visiting El-Mostafa bee

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house, Elmarg, cairo, Egypt; to get natural bee (Apis mellifera) venom therapy for various diseases. The patient is exposed to sting for two weeks gradually to avoid allergic hazards as follows: one sting on the 1st day, two stings after two days, 4 stings after another two days, another 4 stings after one day, 8 stings after another day and finally 16 stings on the 13th day. **The study was approved by the Ethics Board of Al-Azhar University.**

Collections of samples: Blood samples (24) were collected from these volunteers, before and after honey bee sting, at the laboratory of Egypt Air Hospital, Cairo, Egypt following the standard protocol. Samples were collected from September 2014 until December 2015. Consent was taken from all patients before blood sampling. There were two groups of samples:

Group A: samples before sting for 12 patients (5 of these patients were females and the remaining 7 were males, their age ranged from 19 to 63 years).

Group B: samples from the same 12 patients after being exposed to sting.

A sample of blood consisting of 5 ml was obtained from standard radial vein by a sterile disposable syringe from each patient at the Egypt-air hospital, Cairo, Egypt. Each blood sample was poured into clean test tube without anticoagulant but contains gel and clot activator, then centrifuged at 4000rpm for 6-10 minutes. The serum was separated (1 ml) and collected into label multiple clean eppendorf tubes with patient full information, then stored at -20°C until use to test CRP and RF. About 100 µl from each sample is divided into another eppendorf tube and transferred on ice to the immunoparasitology laboratory of Zoology department, Central laboratories building, Faculty of Science for boys, Al-Azhar University, Madinat-Nasr, Cairo, Egypt to test for Complement C3 and C4.

Levels of C3 and C4 by Radial immunodiffusion (RID) plate

Levels of C3 and C4 were measured according to the standard procedure provided with the kits supplied from Biocientifica S.A. Argentina. Radial Immunodiffusion (RID) plates were used for determination of Immunoglobulin and other proteins in biological fluids [11].

Levels of CRP in Serum: C - reactive protein (CRP) was measured by auto analyzer (Dimension® EXEL, Siemens, Germany) using C - reactive protein extended range (RCRP) method on the Dimension ® clinical chemistry system (an in vitro diagnostic test intended for quantitative determination of CRP in human serum). For details of this processing, refer to Dimension® Oprator's Guide.

Levels of RF in Serum by RF Latex: Rapid latex agglutination test for qualitative screening and semi-quantitative determination of rheumatoid arthritis factor, known as RA or RF (anti-gamma globulins in serum) intended for use (BIOSTC Company, El-Mohandseen, Giza, Egypt).

Principle: Due to the presence or rheumatoid factor in the serum, the latex suspension changes its uniform appearance and clear agglutination becomes evident. This change occurs because the RF present in the serum reacts with the Immunoglobulin G (IgG) coated to the latex particles, starting the formation of a web between them. The presence of agglutination indicates a positive result. The absence of agglutination indicates a negative result. Semi-quantitative procedure was executed on positive samples.

Statistical analysis: Significant difference between treatment's means was determined by student t-test where $P<0.05$ were considered statically significant.

RESULTS

Tested values of C3, C4, CRP and RF for All cases before and after exposure to bee venom are shown in Table 1. Patients are complaining from various disease types two cases with RA; two cases with Diabetes Mallets and Arthritis; also two cases with only Arthritis; one case with Back pain; one case with Sebaceous cyst; one case Rheumatic fever; one case gastritis; one case Osteoarthrosis and one case Hepatitis C Virus. Percentages for abnormal values are shown in Fig 1: In 25% of subjects (12 cases), C3 was abnormal and became all normal after sting. C4 was abnormal in 42% of subjects and increased to 50% of subjects after sting. CRP was elevated in 50% of subjects and decreased to 25% of subjects. RF was positive in 17% of cases and became all negative after sting.

Table (1): Showing all studied subjects, the type of disease for each patient and the levels of C3, C4, CRP and RF before and after exposure to sting by honey bee workers.

Subject	Before sting				After sting				Disease type
	C3 80-160	C4 20-40	CRP 0-0.5	RF+ ≥ 8	C3 80-160	C4 20-40	CRP 0-0.5	RF+ ≥ 8	
	mg/dl		IU/ml		mg/dl		IU/ml		
1	107.4	23	1.5	+	125.5	17.4	0.3	-	Rheumatoid Arthritis
2	131.8	15.3	0.5	-	113.3	13.3	0.7	-	Back pain
3	138.2	18.6	0.5	+	138.2	14.3	0.4	-	Rheumatoid Arthritis
4	144.7	34.5	2.1	-	107.4	17.4	1.7	-	Diabetes Mallets, Arthritis
5	68.4	10.4	0.4	-	101.5	8.6	0.2	-	Arthritis
6	131.8	26.7	0.3	-	138.2	23	0.1	-	Rheumatic fever
7	144.7	26.7	0.6	-	125.5	20.7	0.4	-	Diabetes Mallets, Arthritis
8	171.7	40.1	0.2	-	113.3	40.1	0.2	-	Arthritis
9	119.4	46.1	0.1	-	144.7	23	0.1	-	Sebaceous cyst
10	138.2	26.7	0.4	-	138.2	25.4	0.3	-	Gastritis
11	138.8	34.5	2.5	-	90.2	26.7	1.8	-	Osteoarthrosis
12	193.1	31.8	0.2	-	107.4	20.7	0.3	-	Hepatitis C Virus

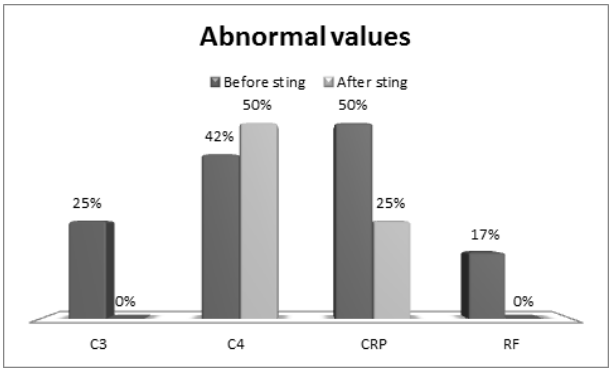

Figure (1): Showing percentage of abnormal values of C3, C4, CRP and RF before and after exposure of the 12 studied subjects (having various disease types) to sting by honey bee workers.

In **Rheumatoid Arthritis** cases: C3 was normal before (107.4 mg/dl; 138.2 mg/ dl) and after sting (125.5 mg/dl; 138.2 mg/ dl); C4 was mostly abnormal before (23 mg/dl; 18.6 mg/dl) and after sting (17.4 mg/dl; 14.3 mg/dl); CRP was elevated (1.5 mg/dl; 0.5 mg/dl) and became normal after sting (0.3 mg/dl; 0.4 mg/dl) and RF was positive and became negative (1st and 3rd cases in Table 1, Fig 2).

In the Back pain case: C3 was normal before (131.8 mg/dl) and after (113.3 mg/dl) sting; C4 was abnormal before (15.3 mg/dl) and after (13.3 mg/ dl) sting; CRP was normal (0.5 mg/dl) and became elevated (0.7 mg/dl) after sting and RF was still negative after sting (2nd case in Table 1, Fig 2).

In Diabetes Mallets, Arthritis cases: C3 was normal; C4 was normal (34.5 mg/dl; 26.7 mg/dl) before and becam abnormal (8.6 mg/dl; 20.7 mg/dl) after sting; CRP was mostly elevated (2.1 mg/dl; 1.7 mg/dl and 0.6 mg/dl; 0.4 mg/dl) and RF was negative (4th and 7th cases in Table 1, Fig 2).

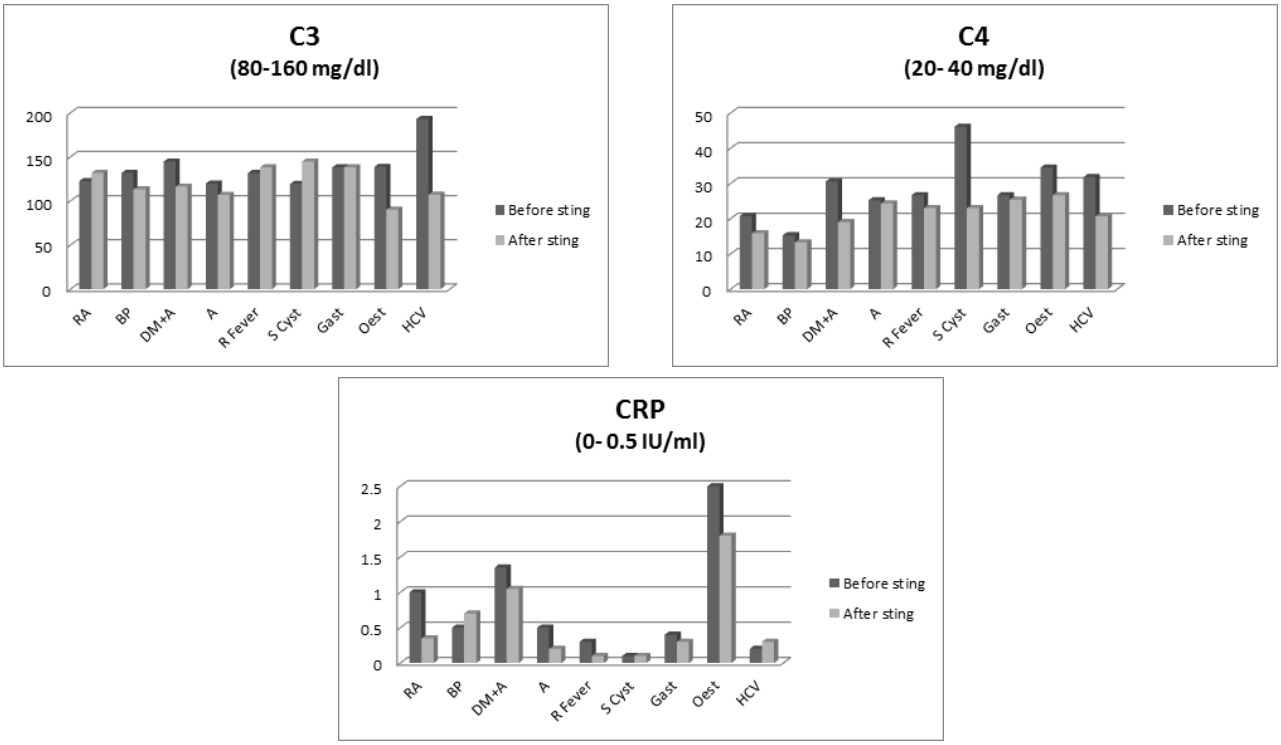

Figure (2): C3, C4 and CRP in patients with various diseases types before and after being sting by honey bees. RA= Rheumatoid Arthritis; BP= Back pain; DM+A= Diabetes Mallets, Arthritis; A= Arthritis; R Fever= Rheumatic Fever; S Cyst= Sebaceous cyst; Gast= Gastritis; Oest= Osteoarthrosis; HCV= Hepatitis C Virus.

In the **Arthritis cases:** C3 was abnormal (68.4 mg/dl; 171.7 mg/dl) and bacame normal (101.5 mg/dl; 113.3 mg/dl) after sting; C4 was abnormal before (10.4 mg/dl; 40.1 mg/dl) and after (8.6 mg/dl; 40.1 mg/dl) sting; CRP was normal and RF was negative (5th and 8th cases in Table 1, Fig 2).

In the Rheumatic fever and in the Gastritis cases: C3; C4 and CRP were normal and RF was negative before and after sting (6th and 10th cases in Table 1, Fig 2).

In the Sebaceous cyst case: C3 was normal; C4 was abnormal (46.1 mg/dl) before and became normal (23 mg/dl) after sting; CRP was normal and RF was negative (9th case in Table 1, Fig 2).

In the Osteoarthrosis case: C3 and C4 were normal; CRP was elevated before (2.5 mg/dl) and after (1.8 mg/dl) sting and RF was negative (11th in Table 1, Fig 2).

In the Hepatitis C Virus case: C3 was abnormal (193.1 mg/dl) and bacame normal (107.4 mg/dl) after sting; C4; CRP were normal and RF was negative (12th case in Table 1, Fig 2).

DISCUSSION

The current work is the first to study the effect of bee venom on serum C3 and C4 in relation to various diseases. Only one case report by ***Song*** [12], in Korea, measured levels of C3 and C4 in a female that developed systemic lupus erythematosus (SLE) after she has 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.

In the present study, the increase and decrease in the levels of complement proteins C3 and C4 indicated activation of the complement system in the patients. The abnormal levels of C4 indicate the classical pathway activation of the complement system, when C3 is either normal or abnormal. The abnormal levels of only C3 when C4 is normal indicate alternative pathway activation of complement.

Kosmas [13] explained that the complement proteins are part of humoral defense and they have the characteristic of interacting with certain antibody molecules once these have combined with antigen. The classic complement pathway is activated by either antibody-coated targets such as microorganisms or antigen-antibody complexes, while the alternative complement pathway is activated directly by bacterial polysaccharides.

C-reactive protein (CRP) is a well-known inflammatory marker. CRP is known to be able to activate complement component [8]. CRP binds to phosphocholine expressed on the surface of damaged cells, as well as to polysaccharides and peptosaccharides present on bacteria, parasites and fungi [17]. This binding activates the classical complement cascade of the immune system and modulates the activity of phagocytic cells, supporting the role of CRP in the opsonization (i.e. the process by which a pathogen is marked for ingestion and destruction by a phagocyte) of infectious agents and dead or dying cells [14,15].

Rheumatoid arthritis (RA) is a chronic, destructive inflammatory disease characterized by the release of numerous proinflammatory Rheumatoid factors (RF). ***Gorevic***[16] has shown in a review article potential association of RF and the classical activation of complement.

In the present study abnormal values for C3 are measured in 25% of cases; 42% of cases for C4; 50% of cases for CRP and 17% of cases for RF. These explain activation of complement through both of classical and alternative pathways. The data of the current research also explain a relation between elevated levels of CRP and RF and activity of the complement. After being exposed to sting by honey bees, the 25% of abnormal C3 turned into its normal levels but the abnormal levels of C4 rose from 42% to 50%. The elevated 50% of CRP dropped into 25% and the 17% positive RF became all negative after exposure of subjects to sting. The current study suggests that the complement activity was only through classical pathway after sting due to the drop in the levels of CRP and RF.

The current work shows that in the Rheumatoid Arthritis cases: Complement was activated through only the classical pathway Since C4 was abnormal before and after sting while C3 was normal. Although the elevated levels of both CRP and RF have droped after sting, the complement remained active. In the Back pain case: complement was also classically activated although CRP and RF were in normal levels before sting and only CRP has elevated after sting. In the Diabetes Mallets, Arthritis cases: Complement was classically activated only after sting. Although RF was normal, CRP was elevated before and after sting. In the Arthritis case: Complement was activated by both classical and alternative pathways before sting because C3 and C4 were abnormal. After sting, Complement activation was only classically because C3 bacame normal. Activation of Complement was not related to either CRP or RF because they were normal before and after sting. In the Rheumatic fever and in the Gastritis cases: No activation of Complement was noticed since C3; C4; CRP and RF were normal before and after sting. In the Sebaceous cyst case: the classically active complement became inactive after sting although both CRP and RF were normal. In the Osteoarthrosis case: Complement was inactive although CRP was elevated and became normal after sting while RF was negative. In the Hepatitis C Virus case: Complement was active through alternative pathway and bacame inactivel after sting while C4; CRP and RF were normal.

The present results were in agreement with the findings of ***Nam*** [17], they have concluded that Bee venom has an anti-inflammatory effect stronger than n-hexane and ethyl acetate. Also, ***Dandona*** [18]; ***Diamanti-Kandarakis*** [19] and Morin-Papunen20 found that increased levels of CRP can be adjusted by treating rats with honey bee venom for 14 days.

The current results were also in agreement with the findings of Abdel-Rahman8 who has found that bee venom has an anti-inflammatory activity by inhibiting RF. Many other researchers have also discussed an association of RF with activation of Complement [21,22,23].

The antioxidant activity was associated with anti-arthritis effect of bee venom, suggesting that the anti-arthritis effects of bee venom might be related to its anti-inflammatory and antioxidant effects. These findings recommend that bee venom serve as an effective therapy for rheumatoid arthritis [24]. Also, ***Kwon*** [25] demonstrated that bee venom can be used for the treatment of multiple conditions including rheumatoid arthritis, tuberculosis, and multiple sclerosis and is also used as the basis for venom immunotherapy.

CONCLUSION

The present study comes to a conclusion that: The complement system has been activated, in patients, by both classical and alternative pathways before treatment with bee venom where it became either only classically activated after treatment or in active. Improved values of both CRP and RF indicate reduction in the inflammatory immune response after exposure of patients to honey bee venom.

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