

The effects of honey on phagocytic activity against *Staphylococcus aureus*

تأثير استخدام العسل على فعالية البلعمة ضد بكتريا المكورات الذهبية

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Abstract

Bee honey is a natural substance reported to stimulate the human immune system. this study aimed at evaluating the in vitro effects of bee honey in stimulation the phagocytosis against *S.aureus* .this study included tow human blood groups ,bee honey treated group and control blood group, tow blood groups are inoculated with *S.aureus* bacteria , after 1hr and 30 min incubation at 37c°, phagocytic activity towards *S.aureus* was assessed by determining the number of ingested *S.aureus* phagocytes per 100 cells (phagocytes and non phagocytes). The results revealed that bee honey at 1% concentration significantly enhanced the phagocytic activity .

الخلاصة

يعتبر العسل احد المركبات الطبيعية المعروف بفوائد عدة منها قابليته على تحفيز الجهاز المناعي للإنسان , ومن خلال هذه الدراسة تم تقييم مدى تأثير العسل على عملية البلعمة ضد بكتريا المكورات الذهبية بمقارنة البلعمة للبكتريا في مجموعتين من دم أشخاص سليمين . حيث يضاف العسل للمجموعة الأولى بينما اعتبرت المجموعة الثانية سيطرة وحققت المجموعتين ببكتريا المكورات الذهبية وبعد فترة حضانة عند درجة 37 م° ولمدة ساعة ونصف تم حساب النسبة المئوية للبلعمة بحساب أعداد الخلايا المبلعمة للبكتريا لكل 100 خلية مبلعمة وغير مبلعمة , وبينت النتائج بان عينات الدم المعاملة بالعسل أظهرت زيادة معنوية في عملية البلعمة للبكتريا مقارنة بمجموعة السيطرة.

Introduction:

Staphylococcus aureus infections constitute an important clinical problem, because of their ability to cause a number of devastating complications and their increasing resistance to current antibiotics (1). Serious complication from hospital *S. aureus* infections may include : Bacteremia (blood infection), Osteomyelitis (bone infection) Endocarditis (infection of the inner lining of the heart and its valves),Abscesses in internal organs such as the lungs and Toxic shock syndrome (2). mortality due to complications of *S.aureus* infections are relatively high especially those caused by methicillin-resistant *S.aureus* ,which had been largely confined to hospitals and long term care facilities (3).

(4) stated that more than 95 percent of patients worldwide with *S. aureus* infections no longer respond to first-line antibiotics (penicillin or ampicillin). Bacteria when invading host tissue are first and fore most exposed to phagocytes but *S.aureus* can avoid phagocytosis by hiding the antigenic surface through production a component (coagulase) which clots fibrin on bacterial surface which is seen as self by the host phagocytes and immune system , therefore phagocytes cannot recognize bacteria upon contact and the possibility of opsonization by antibodies , and the phagocytosis is minimized (5).Honey is a natural products contains various vitamins, minerals and amino acids as well as glucose and fructose. It is generally known that honey has antibacterial activity that has been reported to be due to its high osmolarity, acidity and presence of hydrogen peroxide and unidentified substances from floral sources (6) .recent search also shows that honey stimulate human immune system ,(7) stated that the proliferation of peripheral blood B-

lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentrations as low as 0.1% and phagocytes are activated by honey at concentrations as low as 0.1%. Honey at a concentration of 1% also stimulates monocytes in cell culture to release cytokines, tumor necrosis factor (TNF)-alpha, interleukins (IL-1 and IL-6) which activate the immune response to infection(8).

(9) reported that jungle honey have chemotaxic activity for neutrophils while (10) showed that honey provides a supply of glucose which is essential for the respiratory burst in macrophage that produce hydrogen peroxide , the dominant component of their bacterial destroying activity also the acidity of honey may assist in the bacteria destroying action of macrophage as PH inside the phagocytes value is involved in killing ingested bacteria (11;12) .therefore this study is undertaken to examine the effect of honey on human phagocytes activity against *s.aureus* bacteria.

Materials and methods:

This study was carried out in the Faculty Of Science , Karbala University in the period October to March , It consist of following :

1- Test bacterial suspension:

- Preparation of *S.aureus* suspensions (13):*S.aureus* bacteria was isolated from clinical specimens and cultured on manitol salt agar (oxid). Then *s.aureus* cells were harvested from agar plates , washed twice in phosphate buffer saline (PBS), by low speed centrifugation and diluted in PBS , its concentration was adjusted to McFarland tube (1×10^6 CFU /ml) and then kept in 4C° until use.

2- The test bee honey:

- honey sterilization (14): honey sample was taken to the laboratory and filtered with sterile Seitz filter (0.22 pores) connected to an electronically operated vacuum pump . The sterile honey was diluted with distilled water to obtain final concentration 1% , diluted honey sample was dispensed into sterile bottles and kept at room temperature prior use.

3- The test blood:

- specimen collection : blood samples were taken from 32 persons who their age between 20-40 years , 16 samples were incubated with bee honey and 16 samples are used as controls. Blood was drawn from arm with heparinized tube for the phagocytic determination .

4- Phagocytic activity:

In vitro effect of bee honey on phagocytic activity against *S.aureus* (15): 1 ml of fresh blood taken from persons was immediately placed in plain tube with EDTA, 100µl of sterile diluted bee honey and 100µl of *S.aureus* suspension were added to the blood to obtain a final volumetric of 1 bacteria:10 blood . The mixture were incubated in an incubator at 37c° for 1hr and 30 min , then 2-3 drops of blood were spread on clean slide and stained by leishman stain . Phagocytosis of *S.aureus* was measured by determining the number of ingested *S.aureus* phagocytes per 100 of white blood cells (phagocytes and non phagocytes).

Statistical analysis (16): Data are expressed as Mean±SEM. significance calculated by LSD (least significant difference) A probability of $p < 0.05$ was considered statistically significant.

Results:

The present results concerning the effect of in vitro bee honey challenge on phagocytosis of *S.aureus* . bee honey was found to increase significantly the phagocytic activity against *S.aureus* , significance calculated by L.S.D at 0.05 (table 1).

Table 1: Shows phagocytosis percentage for blood samples treated with honey and controlled blood samples.

Types of samples	Phagocytosis %
Honey treated blood samples	a 68.5 ± 3.8
Control blood samples	47.6 ± 5.2

a = Mean ± standard error of mean
L.S.D at 0.05 = 13.47

Figure (1) below graphically shows the phagocytosis % for blood samples with bee honey contrasted with control blood samples . our finding support that bee honey at 1% concentration is stimulated phagocytosis of *s.aureus*.

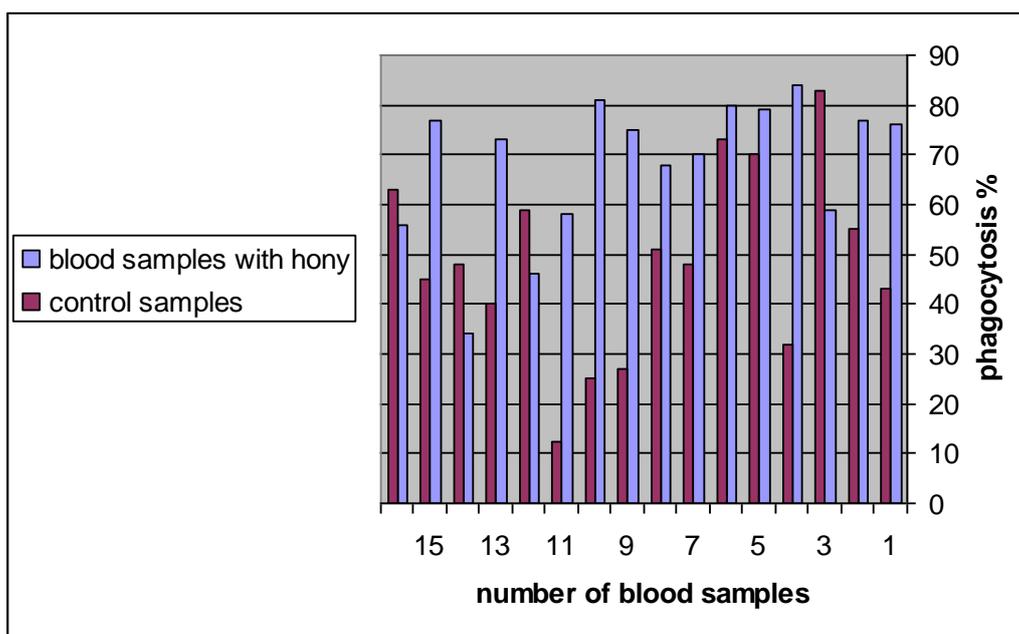


Figure 1: shows the phagocytosis% of *s.aureus* with and without honey.

Discussion:

The incidence of *S.aureus* systemic infections is increasing and because widely using antibiotics in hospitals , these bacteria are acquired resistant to several types of antibiotics like methicillin-resistant *S.aureus* strains which resist all β- lactam antibiotics(17).

The most important first line host defense against *S.aureus* infections is phagocytosis , bee honey was known to stimulate the proliferation of peripheral blood B-lymphocytes ,T-lymphocytes and phagocytes at 0.1% concentration (7) . as evident from the present results , a higher phagocytic activity was recovered from blood samples treated with bee honey at 1% concentration , such result agreed with (8) who stated that honey at 1% concentration stimulate immune response to infection.

This increased phagocytic activity towards *S.aureus* in presence of bee honey may be due to either of the followings : glucose content of bee honey ,the acid PH (between 3-4) which assist in the bacteria destroying action of macrophages (18;19) . the crud honey has a negligible level of hydrogen peroxide which is one of oxygen dependent killing systems of bacteria inside phagocytes (20), but when the honey is diluted the H₂O₂ production is increased by a factor 2,500-50,000 , thus giving slow release antiseptic (H₂O₂) at a level is antibacterial but no tissue damaging (12;21).Thus on conclusion one may state:

The findings of the present study provide further evidence that bee honey stimulate human immune system , manifested by significant increase in the phagocytic activity against *S.aureus*.

Based on the present study and other studies , it is recommended to use bee honey as medical therapy by augmenting the antimicrobial capacity of the host immune system.

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