

EFFECT OF HONEY ON SOMATIC (O) AND FLAGELLAR (H) ANTIBODIES TITER IN TYPHOID FEVER PATIENTS

BY

Ammar Riyadh Qasim

Diyala university, sciences collage, Biology and Microbiology Department

Abstract

This study aimed to determine the honey effect on typhoid fever (TF) patients those which their serum samples assayed and were positive to widal test. In this study, (117) cases male and female with age between (9-50) years, during the period from 30/1 to 1/7 at 2009. Kit- antigen had been used which produced by (Plasmatec Laboratory Product Ltd) to perform widal test by slide method. The results showed that there were (100) of (117) were positive to widal test. Positive cases were divided to two equal groups. First group were treated by effective antibiotics against salmonella species bacteria that cause TF. While second group were given honey in addition to antibiotics treatment for three weeks. Then, the second test was performed for both groups. The results showed that the percent of patients those treated by antibiotics only and had been response (decreasing in titer of antibodies) were (30%) & (56.3) for both somatic (O) and flagellar (H) antigens, While the cases those not response to treatment were (70%) & (43.7%) for both (O) and (H) antigens. The second groups those were treated by antibiotics in addition to honey. Percent of patients those had been response were (68.7%) & (76.2%) for both (O) and (H) antigens. While the cases those not response were (31.3%) & (23.8%) for both (O) and (H) antigens. From these results, the effect of honey with antibiotics in treatment of typhoid fever patients fever was very clear.

Key words: *Honey effect; somatic (O) and flagellar (H) antigen; typhoid fever patients.*

الخلاصة:

هذه الدراسة تهدف الى تحديد تأثير العسل على المرضى المصابين بحمى التيفوئيد والذين تم فحص عينات دمهم وكانت النتيجة ايجابية لفحص الويدال. شملت الدراسة (117) حالة من كلا الجنسين تتراوح اعمارهم من (9-50) سنة خلال الفترة من 1/30 ولغاية 7/1 عام 2009 في ناحية خرنابات. تم استعمال عدة الفحص (kit-antigen) المنتجة من قبل شركة (Plasmatec Laboratory Product Ltd) الانكليزية لإجراء فحص الويدال بطريقة الشريحة المتراسة. اظهرت النتائج (100) حالة موجبة للفحص من مجموع (117). تم تقسيم الحالات الموجبة الى مجموعتين متساويتين، المجموعة الاولى تم علاجهم بواسطة استعمال المضادات الحياتية الفعالة ضد الاصابة ببكتريا salmonella المسببة للاصابة بهذه الحمى، أما المجموعة الثانية فقد تم إعطائهم العسل لمدة ثلاثة اسابيع بالاضافة للمضادات الحياتية و بعدها تم إجراء الفحص الثاني لكلا المجموعتين. أظهرت النتائج إن نسبة المرضى الذين كان لديهم استجابة (انخفاض في عياريه الاجسام المضادة) عند العلاج بالمضادات الحياتية فقط هي (30%) و(56,3%) ولكلا المستضدين الجسمي (O) والسوطي (H) وعلى التوالي. أما الحالات التي لم تظهر استجابة فكانت (70%) و(43,7%) ولكلا المستضدين (O) و(H) وعلى التوالي. اما المجموعة الثانية التي تم علاجهم بالمضادات الحياتية مع العسل، كان هناك (68,7%) و(76,2%) من المرضى الذين كان لديهم استجابة ولكلا المستضدين (O) و(H) وعلى التوالي. اما الحالات التي لم تظهر استجابة للعلاج فكانت (31,3%) و(23,8%) ولكلا المستضدين (O) و(H) وعلى التوالي. ومن خلال النتائج تبين مدى تأثير العسل مع المضادات الحياتية في معالجة مرضى حمى التيفوئيد.

Introduction

Typhoid fever (TF) is a systemic infectious disease characterized by an acute illness, the first typical manifestations are fever, headache, abdominal pain, relative bradycardia, splenomegaly, and leukopenia ^{[1][2]}. *Salmonella enterica* subsp. *enterica* serotype *typhi* is the etiological agent of TF. The infection is an important cause of morbidity in many regions of the world, with an estimated 12 to 33 million cases occurring annually ^[3]. Cases are more likely to be seen in areas like India, South and

Central America, and Africa with rapid population growth, increased urbanization, and limited safe water, infrastructure, and health systems. In recent years, cases have been reported from Eastern Europe ^[4]. Although TF has practically disappeared from developed nations, it remains a serious public health problem in several Asian regions of the former USSR and in parts of South and South-East Asia, Africa and South America ^{[5][6]}.

The definitive diagnosis of TF requires the isolation of *Salmonella enterica* serotype *typhi* from the patient. Cultures of blood, stool, urine, rose spots, blood, and gastric and intestinal secretions can all be useful for diagnosis. Bacteria can be isolated from blood in 73 to 97% of cases before treatment ^[2]. However, in our country, since (i) patients often receive antibiotics prior to medical diagnosis, (ii) bacteria can be isolated from the blood cultures in only 40 to 60% of the cases ^{[7][8][9]}, and (iii) culture facilities may not be available, serologic analysis becomes more important. Widal agglutination test has been widely used in diagnosis of TF in Iraq.

Antibiotics, such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, Amoxicillin and ciprofloxacin, have been commonly used to treat TF in developed countries [10][11]. Ceftriaxone is the best choice for children [12][13]. Aminoglycosides are clinically ineffective in treatment of TF [14]. In some studies it has been shown that Azithromycin (500mg P.O qd for 7 days) as effective as chloramphenicol and given to patients with chloramphenicol susceptible infections [15][16][17].

During Enteric fever, the digestive system is affected and it does not function properly. If some honey is given to the patient frequently, it has a soothing effect on the intestines and the patient does not become weak, Honey gives energy to the patient and also provides treatment. Honey was used to treat infected wounds as long ago as 2000 years before detection of bacterial infection. In c.50, Dioscorides described honey as being "good for all rotten and hollow ulcers" ^[18]. In more recent studies, honey has been reported to have an inhibitory effect to around sixty species of bacteria including aerobic and anaerobic, gram-positives and gram-negatives ^[19]. An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium* ^[19], as well as all the common dermatophytes ^[20]. The current prevalence of antibiotic-resistant microbial species has led to a reevaluation of

the therapeutic use of ancient remedies, including honey ^[21]. This study was performed to detect the effect of honey used with antibiotics in treatment of TF patients.

Materials and methods

This study included (117) patient serum samples male and female which have clinical signs of TF in Khernabat city, (100) patients samples showed a positive result for Widal test, with age between (9-50) years, during the period from 30/1 to 1/7 at 2009. The positive samples were divided into two equal groups. First group included (50) patients treated with antibiotics only. Second group included (50) patients treated with antibiotics and honey. All patient serum samples were tested firstly by using of slide agglutination method Widal test and then secondly after three weeks to detect the effect of honey, through this period patients were treated with antibiotics (in first group) and with /or without honey (in second group).

Patients with TF those diagnosed by Widal test under physician order were characterized by a sustained fever more than 39°C, profuse sweating, headache, unappetite, gastroenteritis, vomiting and non bloody diarrhea, rose-colored spots may appear. Widal test was used to demonstrate the presence of somatic (O) and flagellar (H) agglutinins to *Salmonella typhi* in patient's serum using suspensions of these antigens. A rapid slide test considered now as the most commonly used technique in local laboratories because of its convenience and short time consuming.

Kits of (Plasmatec Laboratory Product Ltd) contain a suspension of H and O antigens in phenol saline. Qualitative test: One drop each of undiluted patients' serum samples for the two antigens are placed on the circled card and one drop of each of *Salmonella* antigens are added separately and gently rotated for one minute. Appearance of agglutination gives qualitative positive results.

Antibiotics treatment was given under physician order as following: in age group (10-15 years old), third generation of cephalosporin (cefotaxime) 500mg as intravenous injection (twice daily) was given for 3 days, then treatment continued with orally ciprofloxacin 500mg which was given for 11 days. In more than 15 years old patients, the antibiotic cefotaxime that given intravenously was of 1gm (twice daily).

Ten milliliters of honey (one table spoonful) was taken by patients every day before breakfast, for three weeks. After this period, the second test was performed.

Results and Discussion

Table (1): Number and percentage (%) of typhoid fever patients and their age groups

Age groups	Number	Percentage of patients
1-10	5	5%
11 – 20	45	45%
21 – 30	34	34%
31 – 40	12	12%
41 – 50	4	4%
Total	100	100%

Table (1) referred to the distribution of patients according to the age groups. More of the infected cases were at (11-20) and (21-30) years which were (45%) & (34%) of patients, respectively. While the lowest were at (1-10) and (41-50) years (5%) & (4%) of patients, respectively. That may be due to the smallest number of the patients were included in this study when compared with the other ages [22][23].

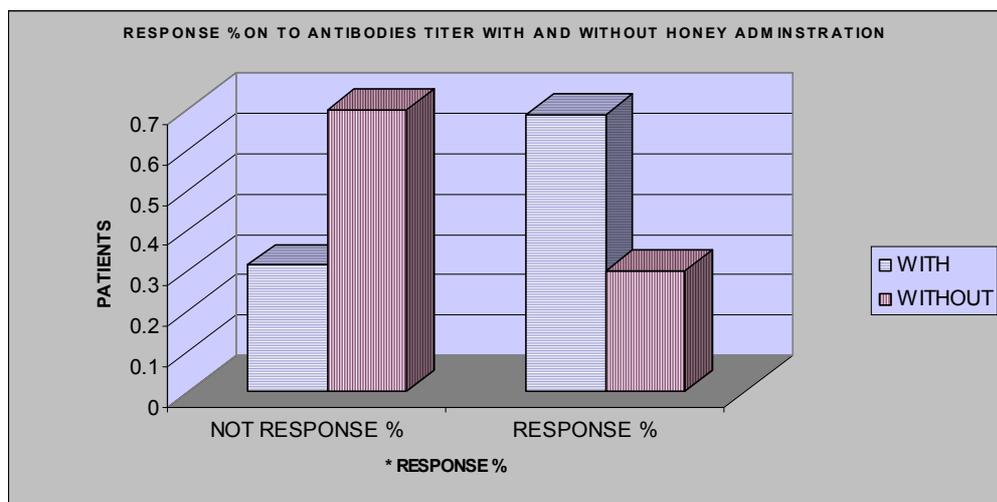
Table (2): Agglutination level against TO, TH in proven cases of typhoid fever

Widal test		1:320	1:160	Negative
*TO	Number	68	30	2
	Percent	68%	30%	2%
*TH	Number	31	43	26
	Percent	31%	43%	26%

*TO: Titer of O agglutinin, *TH: Titer of O agglutinin

Table (2) showed the distribution of infected cases according to the TO and TH agglutinins titer. There were (68%) & (31%) cases had titer of 1:320. The titer 1:160 were in (30%) & (43%) of patients for both TO and TH agglutinins, respectively. There were (2) & (26%) of the proven typhoid cases showed no antibody response to the O and H antigen, respectively. These findings are of paramount significance to clinicians that must often rely solely upon the results of the Widal test in making the diagnosis of typhoid fever, and among that there is a common belief that H antigen is not useful for this purpose. Both of O and H agglutinins are equally important for that purpose. Furthermore, for treatment of typhoid fever patients, data

of the
baselin
e
antibod
y titers
for
typhoi
d
aggluti
nins



should be available [24].

Figure (1): Response percent on TO agglutinin titer with and without honey treatment

* RESPONSE %: % of patients those had any change (decreasing) in typhoid agglutinins titers.

Figure (1) showed the percentage of patients those response (decrease in O antibodies titer) when treated with antibiotics only which were (30%), while the patients percent those treated by antibiotics and honey were (68.7%). Percentage of patients those not response when treated with antibiotics only were (70%), while the patients percent those treated by antibiotics with honey and not response were (31.3%).

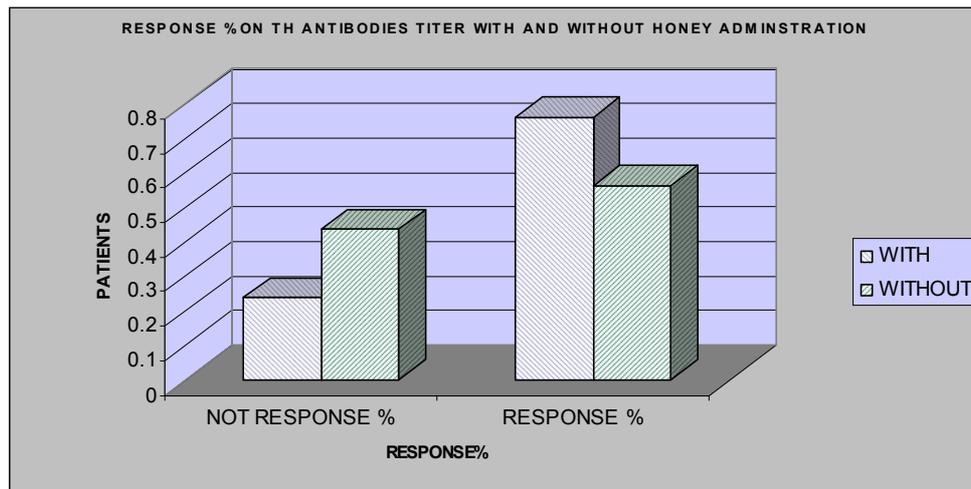
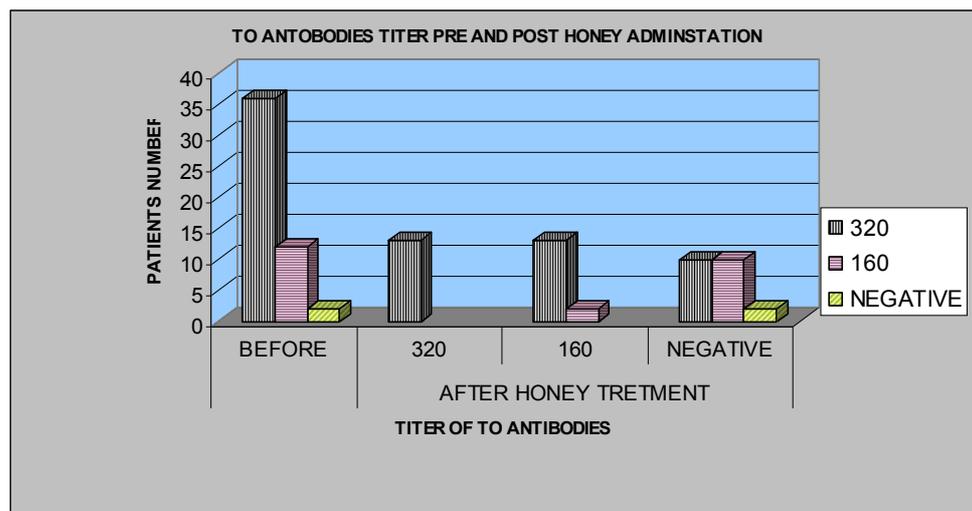


Figure (2): Response percent on TH agglutinin titer with and without honey treatment

Figure (2) showed the percentage of patients those response (decrease in H antibodies titer) when treated with antibiotics only which were (56.3%), while the patients percent those treated by antibiotics and honey were (76.2%). Percentage of patients those not response when treated with antibiotics only were (43.7%), while the patients percent those treated by antibiotics with honey and not response were (23.8%). Figure (1) and (2) referred to the effect of honey administration in antibodies titers decreasing. Horibe et al. (2005), suggested that the changes of serum antibody titers after treatment are related to the suppression of pathogens [25].



NEGATIVE= Negative to Widal test

Figure (3): Typhoid agglutinin O (TO) titer pre and post honey administration

Figure (3) referred to TO antibody titer of (50) cases pre and post-treatment by antibiotics and honey. There were 36 (72%) of (50) patients had TO titer of 1:320. After treatment, antibodies titer changed from (1:320 to 1:160) in 13(36.2%) of the (36) patients, and from (1:320 to Negative) in 10(27.6%). While 13(36.2%) of cases in which antibodies titer still without response. Titer of 1:160 was in 12(24%) of honey treated patients. Titer changed from (1:160 to Negative) in 10(83.3%) of the (12) cases, While there were 2(16.7%) of cases in which antibodies titer still without response.

There were 2(4%) of honey treated patients negative to Widal test somatic antigen at the first diagnosis. Abd-El Aal *et al.* (2007) found that honey had more inhibitory effect (85.7%) on isolated gram-negative bacteria than commonly used antibiotics. A synergistic effect of honey was observed when it was added to antibiotics for gram-negative bacteria and also for coagulase-positive staphylococci [26]. Honey, like other saturated sugar syrups and sugar pastes, has an osmolarity sufficient to inhibit microbial growth [27]. In addition, the pH of honey the glucose content of honey and the acid pH (3-4) may activate the immune system (macrophage) to kill bacteria [28], and release of hydrogen peroxide as antibacterial substances [29]. Honey (at a concentration of 1%) also stimulates monocytes cell to release cytokines, tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activate the immune response [30].

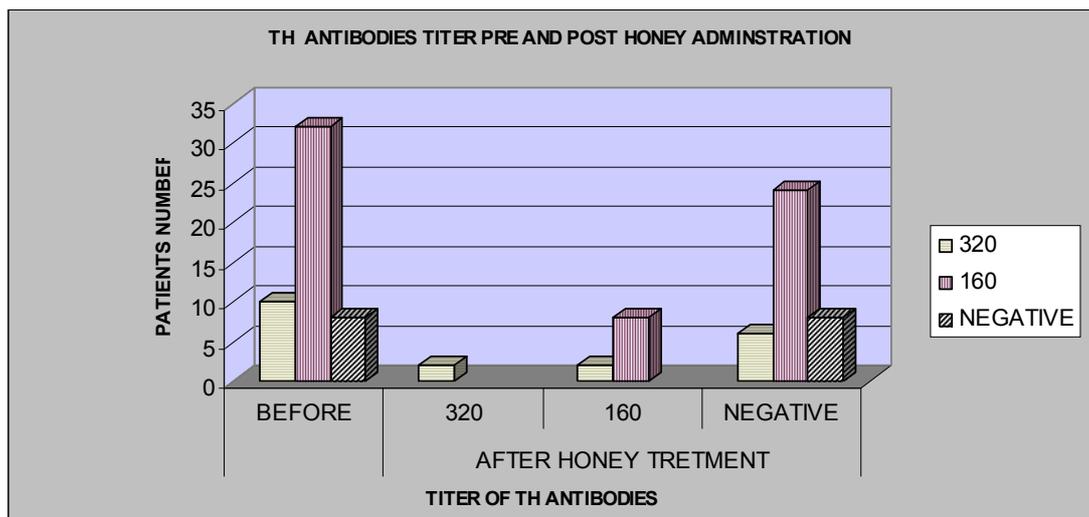


Figure (4): Typhoid agglutinin H titers pre and post honey administration.

Figure (4) referred TH antibody titer of (50) cases pre and post- treatment by antibiotics and honey. There were 10 (20%) of (50) patients have TH titer of 1:320. after treatment, antibodies titer changed from (1:320 to 1:160) in 2(20%) of the (10) patients, and from (1:320 to Negative) in 6(60%) of patients. While 2(20%) of cases in which antibodies titer still without response. Titer of 1:160 was in 32(64%) of honey treated patients, which changed from (1:160 to Negative) in 24(75%) of the (32) cases, While there were 8(25%) of cases in which antibodies titer still without response.

There are 8(16%) of honey treated patients negative to widal test flagellar antigen at the first diagnosis.

Conclusions

- 1- Typhoid fever infect male more than female.
- 2- The response percent (decreasing in titer of antibodies for both (O) and (H) antigens) in patients those treated by antibiotics only, were less than those treated by antibiotics in addition to honey administration.
- 3- The effect of honey with antibiotics in treatment of typhoid fever patients fever is very clear.

Refrences

- 1- Butter, T. (1992). Typhoid fever, p. 1690-1692. *In* J. B. Wyngaarden, L. H. Smith, and J. C. Bennett (ed.), *Cecil textbook of medicine*, 19th ed. W. B. Saunders Co., Philadelphia, Pa.
- 2- Pearson, R. D. and Guerrant, R. L. (2000). Enteric fever and other causes of abdominal symptoms with fever, p. 1136-1150. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), *Principles and practice of infectious diseases*, 5th ed. Churchill Livingstone, New York, N.Y.
- 3- Pang, T., Bhutta, Z. A., Finlay, B. B., and Altwegg, M. (1995). Typhoid fever and other salmonellosis: a continuing challenge. *Trends Microbiol.* 3:253-255.
- 4- Hornick, R. B. (1991). Typhoid fever, p. 803-818. *In* A. S. Evans and P. S. Brachman (ed.), *Bacterial infections of human epidemiology and control*, 2nd ed. Plenum Medical Book Company, New York, N.Y.
- 5- Parry, C., Wain, J., Chinh, N. T., Vinh, H. and Farrar, J. J. (1998). Quinolone-resistant *Salmonella typhi* in Vietnam. *Lancet*;351:1289.
- 6- Mermin, J. H., Villar, R., Carpenter, J., Roberts, L., Samariddin, A. and Gasanova, L., et al. (1999). A massive epidemic of multidrug-resistant typhoid fever in Tajikistan associated with consumption of municipal water. *J Infect Dis*; 179:1416-22.
- 7- Buke, M., Karakartal, G., Gunhan, C., Serter, D., Yuce, K. and Ozkan, F. (1987). Ege Universitesi Infeksiyon Hastaliklari Kliniginde son 10 yilda saptanan tifo ve paratifo olgulari. *Infeksiyon Dergisi* 1:231-236.
- 8- Kalayci, C., Karacadag, S. and Kansu, E. (1987). Typhoid fever—a report of 90 cases. *Infeksiyon Dergisi* 3:89-91.
- 9- Willke, A., Sozen, T., Gultan, H. K., Kurt, H. and Balik, I. (1988). Tifo: 100 hastanın klinik, laboratuvar ve tedavi yönünden değerlendirilmesi. *Ankara Tıp Bulteni* 10:53-62.
- 10- Bhutta, Z. A., Khan, I. A. and Molla, A.M. (1994). "Therapy of multidrug-resistant typhoid fever with oral cefixime vs. intravenous ceftriaxone". *Pediatr Infect Dis J* 13 (11): 990–994.
- 11- Cao, X. T., Kneen, R., Nguyen, T. A., Truong, D. L., White, N. J. and Parry, C.M. (1999). "A comparative study of ofloxacin and cefixime for treatment of typhoid fever in children. The Dong Nai Pediatric Center Typhoid Study Group". *Pediatr Infect Dis J* 18 (3): 245–8.

- 12- Bhulta, Z. A. and Naqvi, S. H., et al. (1991). Multidrug-resistant typhoid in children: presentation and clinical features. *Rev Infect Dis*; 13: 832-836.
- 13- Moosa, A. and Rubidge, C. J. (1989). Once daily ceftriaxone vs. chloramphenicol for treatment of typhoid fever in children. *Pediatr Infect Dis J.*; 8: 696-699.
- 14- Vaudaux, P. and Waldvogel, F. A. (1979). Gentamicin antibacterial activity in the presence of human polymorphonuclear leukocytes. *Antimicrob Agents Chemother*; 16: 743-744.
- 15- Butler, T., et al. (1999). Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicenter trial in India. *J Antimicrob Chemother*; 44: 243-50.
- 16- Thomsen, L. L. and Paerregaard, A. (1998): Treatment with ciprofloxacin in children with typhoid fever. *Natl Med J India*; 11: 226-7.
- 17- Girgis, N. I., Butler, T., Frenck, R. W. and Sultan, Y., et al. (1999) Azithromycin versus ciprofloxacin for treatment of uncomplicated typhoid fever in a randomized trial Egypt that included patient with multidrug resistance. *Lancet*; 354(91730):164-166.
- 18- Gunther, R. T. (1959). *The Greek Herbal of Dioscorides*. New York: Hafner.
- 19- Molan, P. C. (1992). The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee World*; 73(1): 5-28.
- 20- Brady, N. F., Molan, P. C. and Harfoot CG. (1997). The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Pharm Sci*; 2: 1-3.
- 21- Select Committee on Science and Technology. Report no. 7: Resistance to antibiotics and other antimicrobial agents. London: House of Lords, 1998.
- 22- Parry, C.M., Hoa, N.T., Diep, T.S., Wain, J. and Chinh, N.T. (1999). Value single-tube Widal test in diagnosis of typhoid fever in Vietnam. *J. Clin. Microbiol.* 37(9): 2882-2886.
- 23- Gupta. (1994). Multidrug resistant typhoid fever in children; epidemiology therapeutic approach. *Pediatr. Infect. Dis. J.* 13(2) p: 134-140.
- 24- Noorbakhsh, S., Rimaz, S., Rahbarimanesh, A.A. And Mamishi, S. (2003) Interpretation of the Widal test in Infected Children. *Iranian J Publ Health*, Vol. 32, No. 1, pp.35-37.
- 25- Horibe, M., Watanabe, H. and Ishikawa, I. (2005). Effect of periodontal treatments on serum IgG antibody titers against periodontopathic bacteria *Journal*

of Clinical Periodontology Volume 22 Issue 7, Pages 510 – 515 Published Online:
13.

- 26-** Abd-El Aal, A.M., El-Hadidy, M.R., El-Mashad, N.B. and El-Sebaie, A.H. (2007). ANTIMICROBIAL EFFECT OF BEE HONEY IN COMPARISON TO ANTIBIOTICS ON ORGANISMS ISOLATED FROM INFECTED BURNS. *Annals of Burns and Fire Disasters* - vol. XX - n. 2.
- 27-** Chirife, J., Herszage, L., Joseph, A. and Kohn ES. (1983). In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. *Antimicrob Agents Chemother*; 23(5): 766-73.
- 28-** Ryan, G. B. and Majno, G. (1977). *Inflammation*. Michigan: Upjohn.
- 29-** Karayil, S., Deshpande, S. D. and Koppikar, G. V. (1998). Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. [International Journal of Food Microbiology](#), 44(4): 93-6.