The effect of propolis on growth inhibition of *Helicobacter pylori* isolates from peptic ulcer patient

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Abstract:

The aim of this study involved isolation of *Helicobacter pylori*. Mucosal antral biopsy specimens were obtained from 25 patients with peptic ulcer using endoscopic examination from Gastroenterology and Hepatology Hospital. From each patient, two mucosal antral biopsy specimens were taken and we used to detect *Helicobacter pylori* using anti-human IgG and biopsy related test which included rapid biopsy urease test and antral biopsy specimens culturing. The *H. Pylori* isolates were identified by gram stain and biochemical test which included oxidase and catalase tests. The percentage of isolation was 60% and number of isolates was 15 isolates, and evaluating of the inhibitory effect of crud propolis against bacterial isolates. Five graduated concentrations were prepared propolis 12.5, 25, 50, 100, and 200 mg/ml and its activity was checked up by agar well diffusion method. The concentration of propolis exhibit proportionality with zone of inhibition of *H. pylori*. The propolis at concentration 100 and 200 mg/ml was significant activity in comparison with antibacterial used in this study at ($P<0.001$) for each of clarithromycin (100µg), spiromycin (100µg) and trimethoprim (5µg) which were exhibited best activity from each other antibacterial which have been used.

تأثير العكبر في تثبيط نمو جرثومة المَلويَّة البوابية *Helicobacter Pylori* المعزولة من مرضى القرحة الهضمية

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الخلاصة:

هدف الدراسة تضمّن عزل جرثومة الملوية البوابية حيث تم أخذ عينات نسجية من المعدة من 25 مريضاً مصاباً بالقرحة المعدية في وحدة الناظور في مستشفى امراض الجهاز الهضمي والكبد في مدينة الطب. أخذت خزعتان من النسيج المخاطي للمنطقة البوابية للمعدة من كل مريض للتّحري عن جرثومة الملويّة البوابية *H. Pylori* باستخدام عدّة تشخيص الاجسام المناعية IgG وكذلك باستخدام الاختبارات المتعلقة بالعينات النسيجية المتضمنة اختبار انزيم محلل اليوريا السريع وعملية زرع العينات النسجية. شُخِّصتْ العزلات الجرثومة بصبغة كرام وبواسطة الاختبارات الكيموحيوية المتضمنة اختبار الخميرة المؤكسِدة واختبار الكاتاليز المحفِّزة حيث كانت نسبة العزل الجرثومي 60% وعدد العزلات كانت 15

عزلة. وتضمّن تقييم التأثير التثبيطي للعكبر الخام ضد العزلة الجرثومية . حضِّرت 5 تراكيز متدرجة من العكبر الخام (12.5، 25، 50، 100 و 200) ملغم/مل ، وفحصت فاعليتها ضد نمو جرثومة *H. Pylori* بطريقة الانتشار تبين ان هناك علاقة طردية بين تراكيز البروبولس وقطر تثبيط النمو ضد الجرثومة.أظهر البروبولس عند التركيز (100 و200) ملغم/مل فعالية معنوية مقارنة بالمضادات البكتيرية المستخدمة قيد الدراسة عند المستوى ($p<0.001$) لكل من الكلاريثرومايسين (100 µg) والسبايروميسين (100 µg) والترايميثوبريم (5µg) والتي أظهرت افضل تأثير من بين المضادات البكتيرية المستخدمة في هذه الدراسة.

Introduction:

Peptic ulcer disease (gastric and duodenal ulcer) is a common clinical problem. The life time prevalence of peptic ulcer disease is 5% to 10% (1). Gastric ulcers account for about one third of peptic ulcers, and duodenal ulcers account for the rest (2). Peptic ulcer occurs when there is an imbalance between the damaging effects of gastric acid and pepsin, and the defense mechanism that protect the gastric mucosa from these substances. The *H.pylori* is now accepted as major cause of chronic active gastritis, peptic ulcer and associated with development of gastric carcinoma (3). The second major cause of peptic ulcer is the use of non steroidal anti-inflammatory drug (NSAIDs), particularly in the elderly, irritant agent, and environmental factor particularly smoking. For years, the treatment of peptic ulceration centered on measures to neutralize gastric acid, to inhibit its secretion or to enhance mucosal defenses, but recognition of central role of *Helicobacter pylori* revolutionized the approach.

This study concerned with the usage of alternative medicine (epitherapy) which currently used in various countries of the world. The major alternative epitherapy is a propolis (bee glue) which is resinous hive product collected by honey bees from living plants in temperate zones, the source of propolis are the buds of poplar (4). It is important to know the plant source if unsuitable plants are available for the honey bees, toxic substances may be included in the propolis (5).

Materials& Methods:

propolis: The material was purchased from super market in Syria. The material was dried and the ground by an electric grinder. **Antibiotic discs (company: Himedia, Origin: India)**n Spiramycin 100 µg, Amoxicillin 25 µg, Ampicillin 10µg, Oxacillin1µg, Clarithromycin 100 µg, Trimethoprim 5 µg, Metronidazole 5 µg, Gantamicin 10 µg. **Culture Media**: Columbia agar, Brain Heart Infusion Agar, Columbia blood agar base.

Isolation of *H. pylori:*

The first step: By using of one step *H. pylori* test a device which is a rapid chromatographic immunoassay for the qualitative detection of antibodies to *H. pylori* in serum or plasma to aid in

diagnosis of bacteria infection. In this test procedure, anti-human IgG was immobilized in the test line region of the set. The one step *H. pylori* test Device (serum/plasma) has been evaluated with serum and plasma specimens obtained from population of symptomatic individuals who presented for endoscopic examination.

Procedure of the test :(prepared according to the corporation)

Serum or plasma specimen, and/or controls were allowed to reach room temperature (15-30 ◌̊C**)** prior to testing. Placing the Test Device on clean and even level surface. Hold the dropper vertically and transferred 3 drops of serum or plasma (approx. 100 *μL***)** to the specimen well (s) of the test device, and start the time. Avoid trapping air bubbles in the specimen well(s), for colored line (s) to appear, results read at 10 minutes. Do not interpret the results after 20 minutes. Positive: two distinct colored lines appear (C and T). Negative: one colored line appears in the control line region (C). No apparent red or pink line appears in the test line region (T).

The second step

Patient twenty five ages ranged from 25-65 years, were diagnosed as having peptic ulcers (gastric and duodenum) using endoscopic examination was performed every patient with symptoms suggestive of peptic ulcer. Two to three gastric tissue specimens were taken from the antral region of the stomach of each patient during the endoscopic examination, and the time which to take for transport of biopsies and cultivation was about 2-4 hours but not more than 4 hours. The biopsy was transported by sealed box with ice in temperature about 4 ˚C. One biopsy was taken other than before culturing for biopsy urease test.

Biopsy Related Test

A. Biopsy Urease Test: This test was done using commercially available kits. Rapid urease paper test detects *H. pylori* in gastric biopsy (Helicotec UT Plus –strong Biotech corporation-HUP01). That contained a combination of urea and a pH color reagent. *H. pylori* produce large amounts of the enzyme urease, the Helicotec UT Plus test paper detects pH shift and changes color resulting from the breakdown of urea by urease into ammonia. The biopsy was transferred onto test paper after peel the label and re-seal and squeeze, then record the date, time, and patient's information, and then read the result. The benefits of this test are easy to use, easy to store, high sensitivity and specifity, clear, rapid and accurate results.

B. Bacterial Culture Methods

Biopsy specimens for culture were transported to bacteriological laboratory in sterile brain heart infusion broth and were kept in a cool box or 4 ◌̊c for transportation and cultivation through time not more than 4 hours until cultured. The specimens were processed within a limited time of not more than four hours. Gastric biopsies were crushed

on sterile glass slide, homogenized with some drops of same transport media by sterile needles and then cultured on Columbia agar containing 7% defibrinized horse blood, 0.25% yeast extract and campylobacter selective supplement containing polymyxin B 1, 25 iu, vancomycin 5.00 mg and trimethoprim 2.50 mg, per 500 ml medium. The pH was adjusted to 6.8 – 7.2 (6). The plates were incubated in microaerophilic environment created by gas generating Kit (Oxoid – BR 39) in anaerobic humid jar (3-3.5 L) at 35-37 ◌̊ C for up to seven days (7). The colonies of the bacteria were abundant in number relatively, creamy in color and larger in size, about the size of a pinhead. Suspected colonies of *H. pylori* were identified by Gram staining, Catalase and oxidase test. (8).

Part II: Drug Sensitivity Tests

Sensitivity test of 15 isolates of *H. pylori* to eight types of antibacterial drug: clarithromycin(CLR 100 µg), metronidazole (MET 5 µg), amoxicillin(AX 25 µg), gantamicin (CN 10 µg) , oxacillin (OX 1 µg) , ampicillin (AM 10µg),Spiramycin(SP 100µg)and trimethoprim (TMP 5 µg), by using disc diffusion method. Discs containing eight types of antibacterial drugs; clarithromycin, amoxicillin, Spiramycin, oxacillin, ampicillin, gantamicin, trimethoprim and metronidazole, were then distributed carefully on the surface of the inoculated medium, the media were then incubated for 48-72 hours of each antibacterial drugs and measured (mm) the zone of growth inhibition.

Preparation of Standard Dilution of Propolis

The standard dilution of propolis were prepared by using of ethylene glycol as diluents, a good solvent and its inactive against microorganisms growth (9) stock solution of propolis has been prepared and dilution was done into five final concentration respectively (200,100,50,25,12.5 mg/ml).

Testing of Propolis against *H.pylori*

Agar well diffusion method, Perez *et al* (10), was used to assess the general effect of propolis on the growth of *H.pylori.* Inoculated media was poured into several sterile Petri dishes about 20 ml for each plate, Three well were then made on the surface of the medium in each plate by using sterile stainless steel borer. The wells were filled with 0.1ml of different concentration of propolis (12.5, 25, 50, 100 and 200) mg/ml respectively as well as fill 0.1 of ethylene glycol in one of them wells as control. The plates were incubated microearophilically in 35-37 C◌̊ for 3-5 days. The diameters of the inhibitory zones were measured in millimeters.

Statistical Analysis

All the statistical analysis have bee performed by the SPSS 8.0 statistical package. The values between groups have been compared by independent sample – f-test and one- way ANOVA (11)

Result and Discussion:

Sample Description

During the period of the isolation of bacteria from (February 2009 to June 2009) patients attended the endoscopy unit at Digestive and Hepatic Diseases Hospital in Center of Medical country in Baghdad, complaining of suggestive symptoms of peptic ulcer disease like upper abdominal pain, acidity, nausea and vomiting were asked from these patients through the endoscopic process. A total 25 patients with clinical proof of peptic ulcer disease as diagnosed by upper gastro-intestinal endoscopy performed by experienced specialized surgeon have been included in the study. The negative endoscopy results have been excluded.

The First step (Anti-Human IgG Test)

Before endoscopic process, the one step *H. pylori* test device (serum, plasma) for in vitro diagnostic was used. The test has been used for detection of *H. pylori* antibodies in serum or plasma specimen only. Neither the quantitative value nor the rate of increase in *H. pylori* antibody concentration can be determined by this qualitative test. This test has been used as reference method for biopsy culturing. Table (1) shows the numbers of patient (specimen-serum) which have been used for detection of *H. pylori* antibodies (anti-human IgG) and its total percentage was 76% of all positive cases comparison with biopsy related test. Figure 1

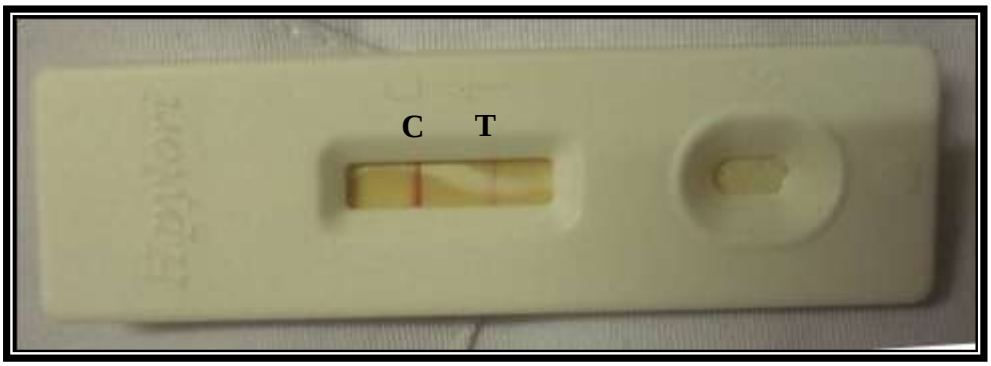

Fig. (1) Shows positive case of anti-human IgG kit for *H. pylori* infection

Several tests, both invasive and non invasive tests, are available to detect *H.pylori* in patients who have been diagnosed with ulcer or who have ulcer symptoms (12,13).

The one step *H.pylori* Test Device (serum) is used in this study before endoscopic process. This is qualitative test based on immunoassay for the detection of *H.pylori* antibodies in serum. The positive percent age of 25 patients for this test were 76%, this indicated the presence of *H.pylori* antibodies but should not be used as sole criteria for the diagnosis of *H.pylori*

infection. Negative results for this test do not at any time preclude the possibility of *H.pylori* infection. In general, the serum levels of anti - *H.pylori* IgG antibodies increased in the presence of infection and could be used as a marker. On the others suggest that, anti- an *H.pylori* IgA antibody *is* less appropriate for this purpose (14).

The Second Step (Biopsy related test):

The result of biopsy related test of the total 25 patient with positive endoscopic diagnosis of peptic ulcer. Fifteen patients (60%) showed positive results for *H. pylori* infection by using of one or more biopsy related test. Table 1.

Rapid Biopsy Urease Test: This test was the most sensitive, capable of detecting 88% of all positive cases. In all positive cases color change started within 5 min. -1 hour of biopsy transferring onto the test paper (kit) Figure 2 shows the kit used for testing urease production and color changes seen in positive cases.

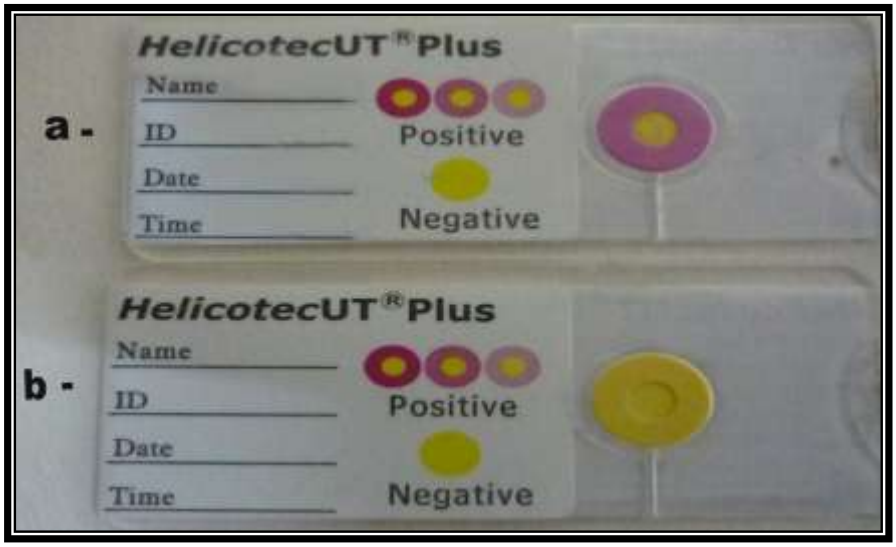

Fig. (2) a-shows positive biopsy urease test
b-shows negative biopsy urease test

Despite some criticism to the invasive techniques as being the universally accepted″ gold standards″ for the diagnosis of *H.pylori* infection, as that infection may be patchy or due to difficulties in culture (15) they still represent the basic techniques for definitive diagnosis. This test remains the simplest, most reliable and rapid test. Sensitivity was 88% of 25 patients were obtained in our study. In which commercially available kit was used rather than the homemade slanted with urea containing agar. This kit is small in size and its preservation for long period of time without the risk of dehydration or contamination (as in home- made urea slants). This made it easy to use by the researcher

in the endoscopy units without help of laboratory for aseptic techniques in culturing. Results were reliable and easy to read. The majority of positive cases show color changes within 5-60 min. from transferring the biopsy onto the test paper indicating the production of large amount of urease enzyme by the bacteria resulting from break down of urea by this enzyme in to ammonia.

Culture Diagnosis: For isolation of *H. pylori,* tissue specimens were plated onto two selective media: The total isolation rate of *H. pylori* was about 60%. Both media had identified 15 positive infections out of 25 patients tested. The negative culture resulted, despite meticulous care, in the whole steps of culturing such as media preparation, transport, incubation, atmosphere and identification steps. The colonies of the isolated bacteria have been abundant in number especially in some culture and very scanty in others Figure 3, the colonies have been creamy in color, and tiny to pinhead in size, and the bacterial contamination of media has been frequent such as fungi. The growth rate of *H. pylori* was slow, in most cases 5 to 7 days have been needed for colonies to appear in 48-72 hour.

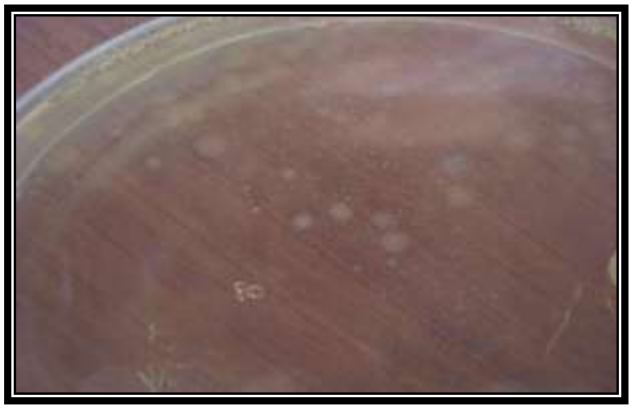

Fig. (3) *H. pylori* colonies after subculture

Table (1) Number of patients with positive to gastric ulcer who were used in this study and tests which were used in detection of *H. pylori*

	NO. Of patients	IgG		Urease		Culture	
		Positive	negative	Positive	negative	Positive	negative
	5	5	0	5	0	4	0
	5	4	1	4	1	3	1
	6	4	2	5	1	3	0
	4	3	1	4	0	2	0
	5	4	1	4	0	3	1
Total	**25**	**19**	**5**	**22**	**2**	**15**	**2**
		76%	**24%**	**88%**	**8%**	**60%**	**8%**

The gold standard for presence of most infectious disease is successful culturing of the microorganism (16). The isolation rate in present study was 60%, the colonies were abundant, moderate in some isolates and scanty in others, creamy and tiny to pinhead in size. Bacterial contamination of the medium was frequent. The growth rate of *H.pylori* on this medium was slow, as 5 to 7 days were needed for the colonies to appear in primary isolation. At present, culture of *H.pylori* from gastric antral biopsy specimens is a reference technique in bacteriology and is essential for drug-susceptibility testing and analysis of virulence factors (17). Although it is usually considered a fastidious, time consumer and expensive procedure, culturing on solid medium is the standard technique used in most laboratories for the isolation of *H.pylori* from gastric biopsy specimens (18).Several factors, which are difficulty to control, cause the difficulty with the culturing of the organism. Patchy distribution of the organism on the gastric mucosa, contamination of biopsy and forceps, presence of oropharengial flora, loss of viability of the organism during transportation, and preparation of media. etc, altogether may be responsible for a poor negative predictive value associated with culture of *H.pylori* (19).These figures were obtained despite careful handling of all culturing steps.

Gram stain of *H. pylori* : All *H. pylori* isolates were subjected to gram staining. The Characteristics of the bacteria have been observed as gram negative, spiral shaped rods Figure(4).

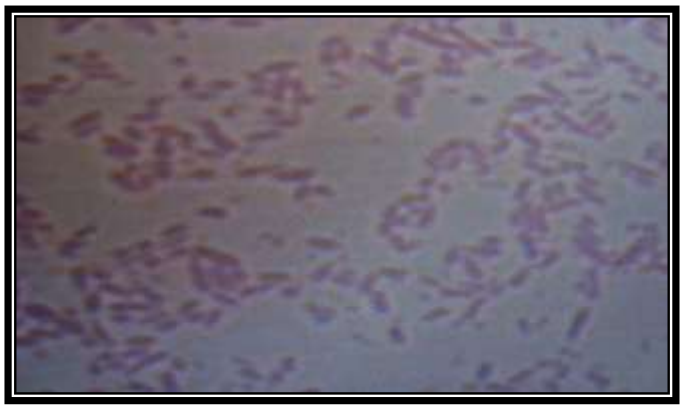

Fig. (4) Gram stain of *H. pylori* isolated from patient with peptic ulcer

Biochemical Tests: Two addition biochemical tests have been performed to confirm the identity of *H. pylori*. These two tests were Oxidase and catalase, which were performed on all isolates. Both tests were positive for all 15 isolates. Table (2) and Figure (5).

Table (2) results of biochemical tests on *H. pylori*

Biochemical tests	*H. pylori* isolates	
	Positive reaction	Negative reaction
Oxidase	15 (100%)	0
Catalase	15 (100%)	0

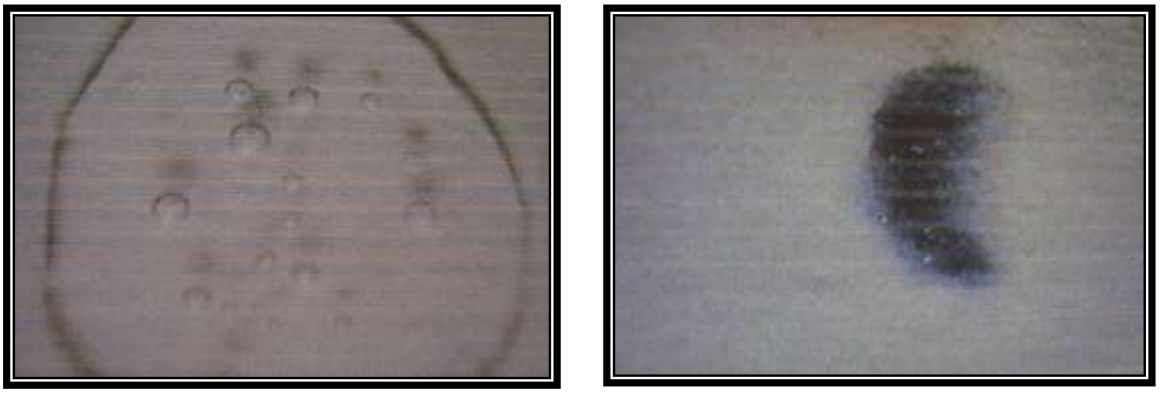

Fig. (5) a- positive Catalase b- positive Oxidase

Peptic ulcer disease is a common chronic inflammatory condition of the stomach and duodenum. In the United States peptic ulcer affects as many as 10% of people at some time in their live (20). In UK, peptic ulcer affects about 6-13% of men and 2-5% of women between 15 and 64 year of age (21) Although this condition has a relatively low mortality, it results in substantial human suffering and financial expense.

Formerly, peptic ulcer was considered a chronic disorder of unknown etiology; stress, irritant, spicy food, excess stomach acid and the use of non steroidal anti-inflammatory drugs (NSAIDs) like aspirin, or).any combination of these factors were considered as the causes of peptic ulcers. The discovery of *H.pylori* by Warren and Marshall in 1983 has changed the conventional concept of gastro-duodenal ulcer disease (22). Many studies suggest a high correlation between *H.pylori* infection and peptic ulceration, it is reported that 60-70% of patients with gastric ulcer, and 90-95% of patients with duodenal ulcer have marked gastric colonization of *H.pylori* (23). In UK, half of those over 50 are infected (Martin, 1999). It seems that the disease of the stomach is multifactorial and infection by *H.pylori* is probably one part of a sequence of events required to produce disease. Other factors are needed; these include hyper secretion of acid, smoking and genetic predisposition (24).The isolation rate of *H.pylori* from gastric biopsy specimens of peptic ulcer disease in this study 60%, which may appear lower than the figures mentioned in some literatures. This may be due to

despite meticulous care in the whole steps of culturing including careful media preparation, transport, incubation atmosphere and identification steps.

Antibacterial Drugs Susceptibility

The effect of seven type of antibacterial agent on several, high density bacterial growth and abundant colonies of *H. pylori* isolates, Clarithromycin was found to be the most effective against *H. pylori*, mean diameter zone of inhibition was 16.88 ± 0.5mm,($p>0.05$), followed by spiramycin 15.44 ± 0.44mm,($p>0.05$) in comparison with other used antibacterial, Metronidazole was to be the least effective *against H. pylori*, the mean diameter was 10.44 ± 0.32mm, ($p>0.05$). However, ampicillin was ineffective to *H. pylori* isolates at level of significant ($p >0.05$) and ($p<0.01$).Table(3).

Antibacterial	Mean diameter zone (mm)
Clarithromycin 100 µg	16.88 ± 0.505 **A**
Spiromycin 100 µg	15.44 ± 0.444 **B**
Trimethoprim 5 µg	12.22 ± 0.382 **C**
Gantamycin 10 µg	11.22 ± 0.486 **D**
Oxacillin 1 µg	10.77 ± 0.486 **E**
Metronidazole 5 µg	10.44 ± 0.327 **E**
Amoxicillin 25 µg	10.71 ± 0.322 **E**
Ampicillin 10 µg	0.00 ± 0.000 **F**

Table (3) Diameter zone of inhibition of different antibacterial agents against *H. pylori*.

-The values represent Mean ± SE

-Different capital litter refer to significant differences between groups vertically $P<0.05$.

Effect of Propolis with Different Concentration on Growth of *H. pylori*

The effects of different concentrations of propolis on *H.pylori* growth were used. Propolis showed maximum inhibitory effect on all tested isolates, mean diameter of *H. pylori* zones of inhibition by propolis are presented in Table (4). At concentration of 200 mg/ml (100%) showed large considerable diameter of zone of inhibition ≥ 20 (24.33±0.3mm), and at 100 mg/ml 77.77 %($p < 0.05$). However, at concentration 12.5 mg/ml was (10 ±

0.32) ($p < 0.05$). In vitro sensitivity of *H. pylori* established by agar well diffusion method; found that the lowest concentration of propolis which can inhibit the growth of *H. pylori* Table (4).

Table (4) Diameter zone of inhibition of Propolis for different concentrations against *H. pylori*

concentration mg/ ml	diameter zone (mm)	Zone diameter range(mm)	zone diameter ≥15(%)mm	zone diameter ≥20(%)mm
Propolis 12.5	10 ± 0.322 **A**	9 – 12	0 %	0 %
Propolis 25	16 ± 0.533 **B**	13.5 - 18	77.77 %	0 %
Propolis 50	18.66±0.381 **C**	17 - 20	66.66 %	33.33 %
Propolis 100	20.55± 0.367 **D**	19 - 22	22.22%	77.77 %
Propolis 200	24.33 ± 0.3 **E**	23 -26	0 %	100 %
Ethylene glycol	0± 0.000 **F**	0	0%	0%

The comparison of zone of inhibition and activity against of *H. pylori*; between lowest and highest concentration of propolis, with different antibacterial agents has been done. The result exhibited significant at level ($p<0.05$) between lowest concentration of propolis 12.5 mg/ml and Clarithromycin, Trimethoprim and Gantamycin respectively, However, their has been exhibited no-significant with oxacillin and metronidazole.

Although pharmacologically, the relationship between different concentrations of propolis as well as all antibacterial agents were used in this study, and zone of inhibition was done, by drawing standard curve in semi log paper to determine activity of each concentration of antibacterial agents in zone of inhibition of *H. pylori*. Figure(5).

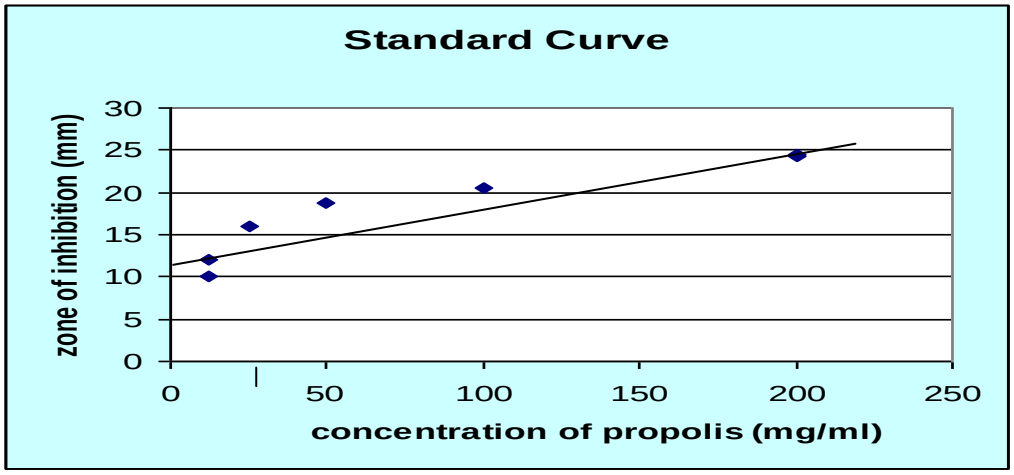

Fig. (5) Proportional relationship between concentration of propolis and the mean diameter zone of inhibition

Although *H.pylori* is sensitive to many antimicrobial drugs *in vitro*, it is difficult to eradicate from the stomach. This may be ascribed to antibacterial breakdown by gastric acid, clearance by gastric emptying, and the difficult-to penetrate mucous layer in which the bacterium resides (25). In different propolis sample, various substance combinations are responsible for the antibacterial activity of the bee glue. In Bulgaria and several Mediterranean countries, propolis contains mainly flavonoids and esters of caffeic and ferulic acids.propolis samples from temperate zone, flavonoid and esters of phenolic acids are known to associate with antibacterial activity. Although the inhibitory effect of propolis on Gram- positive bacteria has been demonstrated, the activity of propolis against *H.pylori* is a matter of controversy (26); for example propolis has shown good activity against *Haemophillus influenzae* and *Moraxella catarrhalis*, but not against Enterobacteriaceae. This fact can explained as Gram negative bacterial isolates have lipopolysaccharide (LPS) in bacterial cell wall which sharing with complex proteins that these capable of to prevent passage of undesirable materials into bacterial cell in compared with gram positive bacteria (27). However, the lipopolysaccharide (LPS) in *H.pylori* has low biological activity as compared to LPS form other Gram negative bacteria(27), which may be explained by the unusual composition of lipid A (15). The anti- *H.pylori* activity of Brazilian propolis has recently been reported, labdane-type Diterpenic and some prenylated phenolic compounds being the main antibacterial substances (5).

In the present study, showed the Propolis with different concentration (12.5, 25, 50, 100, 200) mg/ml has considerable highly activity against *H.pylori* with mean diameter zone of inhibition (10 ± 0.322, 16 ± 0.533, 18.66 ± 0.381, 20.55± 0.367, 24.33 ± 0.3) mm respectively, so the relationship between the activity of propolis against of 15 bacterial isolates and zone of inhibition showed proportionality with the concentration of propolis. This may be attributed to increase the inhibitory effect of active ingredients that these have antimicrobial effect especially the flavonoid, phenolic acid, pinocembrin, caffeic acid, cinnamic acid and pinobanksin (28). The type of propolis which was used in the present study was European propolis. The literature attributes the biological activity of propolis and aromatic acids. In European, for example the propolis is cited as having a large flavonoid content often surpassing 20% (18.19). *H.pylori* resistance to antimicrobial is a growing problem. The reported frequencies of resistance to antibacterial drugs have among subgroups within a study population (4). For example, metronidazole resistance varies from 10 to 80% among geographic regions (12, 14).

In the current study, all antibacterial used show different effect against *H.pylori* isolates except the ampicillin which the *H.pylori* showed resistance against it with all isolates. The *in vitro* efficacy of amoxicillin for *H.pylori* infection is high; however, when amoxicillin is used as a single therapeutic agent, this rate drops to low efficacy at acid pH (22). This is reason why amoxicillin is used in combination with anti-secretory drugs (24). A recent report has identified a number of *H.pylori* strains that are resistant to amoxicillin (19), but none of the isolates in our study showed resistance to this agent.

Kato *et al* (29) studied the primary resistant rates among resistant 625 *H.pylori* isolates from Korea. They reported that 40-60% were to metronidazole and 5-9% to clarithromycin. In their study resistance to metronidazole and clarithromycin increased from 33.3 to 47.7% and 4.8 to 7% respectively). Kato *et al* (29) performed a study on the antibiotic resistance of *H.pylori* strains in Japanese children, it has been reported that clarithromycin resistance results in significantly lower *H.pylori* eradication rates (18). Rates of *H.pylori* resistance to clarithromycin are frequent (30). The estimated range of resistance to this antibacterial in *H.pylori* infected patients in various nation and regions of the world is 1-15% (Alarcon *et al.,* 1999; Ferrero *et al.,* 2000; Street *et al*., 2001). In this study, the rate of *H.pylori* resistance to clarithromycin and metronidazole was 0%, Clarithromycin showed high efficacy against *H.pylori*. However, metronidazole showed less effective antibacterial drug but not resisted by *H.pylori*. This may be due to limited uses of clarithromycin in Iraq and extensive use of metronidazole showed less efficacy. *H.pylori* is most sensitive to clarithromycin *in vitro,* and this agent is extensively used in combination regimens especially for peptic ulcer infection. Oral administration yielded high serum and tissue concentration, and the drug is stable in acid environments. Rate of *H.pylori* resistance to ampicillin was 100 %, this may be due to widespread of ampicillin in Iraq with different manifestation and use it in different disease without physician advices, and therefore different bacteria became resistant to this drug.

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