

## Propolis Use and *Helicobacter pylori*: Two Case Reports

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### Abstract

Propolis possesses many beneficial biological activities and <sup>13</sup>C urea breath (<sup>13</sup>C UBT) test is considered to be the best non-invasive test for the diagnosis of *Helicobacter pylori* infection. The aim of this study was to evaluate propolis effects on *H. pylori* infection status and <sup>13</sup>C UBT results of two untreated symptomatic adults. The first patient with chronic gastritis and gastroesophageal reflux was tested by <sup>13</sup>C UBT for *H. pylori* several times over >1 year. The subject used only non-antibiotic agents involving propolis. All <sup>13</sup>C UBT results of the patient were positive. The second patient had taken propolis on the day before the test and the UBT result was negative, while the culture was positive. In conclusion, although propolis alone or with other non-antibiotic agents may be a good adjunct in the infection therapy, the long-term bee glue use did not lead to *H. pylori* eradication in the first patient. Conversely, the use of propolis several days before the test may result in false negative UBT results, raising the question of the need to avoid propolis use at least several days before the UBT. The optimal dosage and duration of bee glue use should be further evaluated.

**Keywords:** *Helicobacter pylori*; propolis; urea breath test; UBT; non-antibiotic; probiotics

### Резюме

Прополисът притежава много полезни биологични активности и <sup>13</sup>C дихателният урея (<sup>13</sup>C UBT) тест се счита за най-добрият неинвазивен тест за диагностициране на инфекцията. Целта на това изследване е да се проучи действието на прополиса върху статуса на *H. pylori* инфекция и резултатите от <sup>13</sup>C UBT на двама нелекувани симптоматични възрастни. Първият пациент с хроничен гастрит и гастроезофагеален рефлукс е изследван с <sup>13</sup>C UBT за *H. pylori* няколко пъти в продължение на над една година. Пациентът е приемал само неантибиотични агенти, вкл. прополис. Всички резултати от <sup>13</sup>C UBT на този пациент бяха положителни. Вторият пациент е вземал прополис в деня преди теста, а резултатът от UBT беше отрицателен, докато културелното изследване беше положително. В заключение, въпреки че прополисът самостоятелно или заедно с други небиотични агенти може да бъде добър суплемент в лечението на инфекцията, продължителното използване на агента не доведе до ерадикация на *H. pylori* при първия пациент. Обратно, прополисът, използван няколко дни преди началото на теста, може да доведе до фалшиви отрицателни UBT резултати, което поставя въпроса за необходимостта от избягване на употребата на прополис поне няколко дни преди провеждане на UBT. Оптималната дозировка и продължителността на употребата на прополиса трябва да бъдат допълнително проучени.

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## Introduction

*Helicobacter pylori* eradication can strongly reduce the risks of severe gastroduodenal diseases in the positive patients. However, eradication is often difficult to be obtained mainly due to the constantly increasing *H. pylori* resistance to antibiotics and poor patient compliance, patient allergy to the drugs used or side effects of the therapy (Talebi Bezmin Abadi, 2017). Because of this, the use of non-antibiotic agents may be helpful as a treatment adjunct or for prophylaxis of the infection.

Urea breath test (UBT) is considered to be the best non-invasive test for the diagnosis of the infection (Malfertheiner *et al.*, 2012). It is known that using antibiotics up to one month before the test and using proton pump inhibitors up to 14 days before the test as well as the presence of bleeding ulcers or atrophic gastritis can lead to false-negative <sup>13</sup>C UBT results due to reduced bacterial density (Malfertheiner *et al.*, 2012).

The aim of this study was to evaluate the effects of propolis on the *H. pylori* infection status and <sup>13</sup>C UBT results of two untreated adult patients with gastroduodenal diseases.

## Case reports

The case reports of two adult patients with gastroduodenal diseases taking propolis with or without other non-antibiotic agents were described (Table 1). The patients were untreated for *H. pylori* infection and did not take nonsteroidal anti-inflammatory drugs (NSAIDs). Informed written consent was obtained from both patients.

The UBTs were performed following at least a 6-hour fast of the patients. The patients received 2 g of citric acid as a test meal and 75 mg of commercially available <sup>13</sup>C-urea (Gisbert and Pajares, 2004). Two breath samples taken from each subject were a baseline breath sample and a second (post-dose) breath sample exactly 30 minutes after the <sup>13</sup>C-urea ingestion.

The breath samples were evaluated by <sup>13</sup>C UBT infrared spectrometer IR-force 200 (Richenforce, Beijing, China) or HeliFANplus (Fischer ANalysen Instrumente GmbH, Leipzig, Germany). Cut-off delta over baseline (DOB) value of 2.05 was used according to the ROC curve that was previously determined (Boyanova *et al.*, 2013).

The culture was performed with two gastric biopsy specimens as described previously (Boyanova *et al.*, 2005). Briefly, a selective (containing a selective supplement of Dent) and non-selective media (Mueller Hinton agar, Oxoid, UK with 5%

sheep blood) media were used for the isolation and microaerophilic (CampyGen envelopes, Oxoid, UK) incubation was performed at 37° C for up to 10 days.

The study was approved by the Ethical Committee of Medical University of Sofia.

One of the patients (a 60-year-old woman) suffered from chronic gastritis and gastroesophageal reflux, reported to have penicillin allergy and avoided using antibiotic. Three months before the first UBT testing, she used bismuth compound (CBS-colloidal bismuth subcitrate 120 mg bid) for 20 days, as well as propolis (30% ethanol extract at recommended dose of 30 drops tid), lansoprazole (30 mg bid) and probiotic/probiotic combination (containing 2 x 10<sup>9</sup> colony forming units of *Lactobacillus spp.* and *Bifidobacterium spp.* per capsule with inulin addition) daily for several months. The UBT result was positive. Six months later, she underwent a second UBT test and reported that had taken CBS (as above) for 20 days, and both probiotic combination and propolis for two months. The UBT result was positive. Fourteen months after the first UBT test, the patients performed one more UBT test, reporting the use of propolis only (as above). The third UBT result was positive like the previous ones.

The second patient was a 40-year-old man with chronic gastritis. He was untreated for *H. pylori* eradication, and had taken propolis (30% ethanol extract at recommended dose) irregularly, including on the day before the test. The UBT result was negative, while the culture was *H. pylori* positive.

## Discussion

Propolis or bee glue possesses important antibacterial, antiviral, antifungal, antioxidant, and antitumor activities (Khalil, 2006). The resinous mixture contains phenols, including caffeic acid phenethyl ester, as well as terpenes, tannins, polysaccharides, aromatic acids and aldehydes and other compounds (Khalil, 2006). The caffeic acid phenethyl ester is linked to beneficial biological activities such as antioxidant, anticancer, antimicrobial, immune-modulatory and anti-inflammatory effects (Yuksel and Akyol, 2016).

In our previous study (Boyanova *et al.*, 2005), Bulgarian propolis (30% ethanol extract) showed a good and dose-dependent *in vitro* activity against most *H. pylori* strains tested, inhibiting >86% of the strains tested by an agar-well diffusion method. However, Coelho *et al.* (2007) assessed Brazilian green propolis (20 drops of alcoholic extract

**Table 1.** Untreated *H. pylori* positive symptomatic adults in the study.

No	Sex and age (years)	UBT	Non-antibiotic agents	UBT result	Culture (results)
1	Woman (60)	1 <sup>st</sup> month	Propolis <sup>1</sup> , probiotics <sup>2</sup> , PPI <sup>3</sup> and CBS <sup>4</sup> 3 months earlier	Positive	NA <sup>5</sup>
		7 <sup>th</sup> month	CBS for 20 days, propolis and probiotics for 2 months	Positive	NA
		15 <sup>th</sup> month	Propolis regularly	Positive	NA
2	Man (40)	One visit	Propolis on the day before the test	Negative	Positive

<sup>1</sup>Propolis-30% ethanol extract, 30 drops tid

<sup>2</sup>Probiotic/prebiotic combination (2 x 10<sup>9</sup> colony forming units of *Lactobacillus* spp. and *Bifidobacterium* spp. per capsule with inulin addition bid).

<sup>3</sup>PPI- lansoprazole 30 mg bid

<sup>4</sup>CBS- colloidal bismuth subcitrate 120 mg bid

<sup>5</sup>NA-non-available

of propolis tid for seven days) effect on *H. pylori*-positive subjects and observed temporary *H. pylori* suppression in some patients by UBT.

In this study, even long-term use of propolis, without or with other non-antibiotic agents, failed to eradicate *H. pylori* infection. Nevertheless, different dosages or non-antibiotic combinations with bee glue should be further evaluated. On the other hand, propolis use shortly before the UBT led to a false-negative result, while the culture was *H. pylori* positive. This result can pose the question of the need to avoid using bee glue at least several days before UBT testing. Similarly, in the study of Coelho *et al.* (2007), in half of the patients evaluated, propolis led to a 20% reduction of the UBT values, reflecting a temporary decrease in the bacterial density in the stomach.

Cui *et al.* (2013) observed that a propolis phenolic compound (caffeic acid phenethyl ester) reduces *H. pylori* enzyme (peptide deformylase) activity. Recently, Baltas *et al.* (2016) have found that ethanol **propolis extracts inhibited** *H. pylori* urease, with inhibition concentrations (IC<sub>50</sub>) of 0.260-1.525 mg/ml.

Therefore, bee products have a potential in the control of *H. pylori* infection. In our prior study, honey consumption was linked to a lower *H. pylori* positivity rate (odds ratio, 0.38 and a 95% confidence interval, 0.19-0.78), (Boyanova *et al.*, 2015). Moreover, Nostro *et al.* (2006) have reported that a propolis + clarithromycin combination had either synergistic or additive activity against *H. pylori*.

## Conclusion

In conclusion, even though propolis use alone or together with other non-antibiotic agents may be a good adjunct in *H. pylori* therapy, it was not able to eradicate *H. pylori* infection. Conversely, using bee glue shortly before the UBT testing can be a reason for false-negative UBT results. Propolis use in the control of *H. pylori* infection requires additional evaluation to determine the optimal doses, treatment duration and combinations with other non-antibiotic agents. The avoidance of propolis use several days before UBT needs to be further evaluated.

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