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Evaluation of therapeutic properties of *Apis florea* honey against *Helicobacter pylori*

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Abstract
Honey is not only considered as a fabulous food product, but also it has healing properties. Iranian men are susceptible to gastrointestinal cancer caused by *Helicobacter pylori*. It is very imperative to find effective methods to control this bacterium without side effects. In this study *Apis florea* honey samples from 7 various floral and geographical origins of Iran and 5 samples from each region were analyzed in terms of acidity, pH, moisture, sucrose, fructose, glucose, proline, diastase, Ash and HMF. Also, honey samples were analyzed for antioxidant activity using the FRAP, DPPH and ABTS methods. Using atomic absorption measurements, the honey samples were also tested for their phenolic and flavonoid content, protein concentration, and mineral content. Furthermore, antibacterial activity of honey samples against *H. pylori* was assessed in mice's gastrointestinal tracts both in vitro and in vivo. In-vitro antibacterial activities of different honey samples was performed using the agar well diffusion method and In-vivo antibacterial activities of them was analyzed by introducing *H. pylori* into the gastrointestinal tract of mice, feeding them with the honey samples for 75 days and dissecting their stomachs. The Sydney system was also used to assess the degree of inflammation in addition to detecting *H. pylori* contamination. The results showed that the highest and lowest phenol contents were observed in Bushehr (755.83 g/g) and Iranshahr (348.83 g/g), respectively. According to the results, the highest and lowest levels of antioxidant activity with ABTS method, were found in Bushehr (786.40 ± 29.73 µg/g) and Iranshahr (324.90 ± 4.90 µg/g) honey samples. The DPPH and FRAP methods confirmed the results of the ABTS method for honey antioxidant activity. A statistical analysis of the honey samples revealed significant positive correlations between antioxidant and antimicrobial activity. According to the results of antibacterial activity test, in the Bushehr honey sample, gastric mucosa showed mild inflammation and mild degeneration, along with severe eosinophilia. Based on these results, Jujube (*Zizyphus spina Christi*) honey from Bushehr could prevent and cure infections and inflammation caused by the bacteria H. pylori in the gastrointestinal tract.

Materials and methods

Fig 1. Investigation of the physicochemical properties of *A. florea* honey

Fig 2. Evaluation of antibacterial properties of *A. florea* honey against *Helicobacter pylori* in vitro

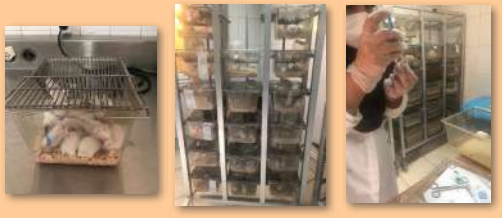

Fig 3. Evaluation of antibacterial properties of *A. florea* honey against *Helicobacter pylori* in vivo

Results

Table 1 - Antioxidant power of honey samples collected from different areas

Sample	Antioxidant capacity (µgr /gr)		
	ABTS (Mean±SE)	FRAP (Mean±SE)	DPPH (Mean±SE)
Dezful	533.44±19.9 ^{c}	23.28±0.80 ^{ab}	424.64±4.63 ^{d}
Iranshahr	324.90±4.90 ^{d}	14.41±0.95 ^{d}	393.03±11.98 ^{d}
Bushehr	786.40±29.73 ^{a}	26.98±0.92 ^{a}	625.20±26.17 ^{a}
Jahrom	691.96±32.38 ^{b}	23.37±1.81 ^{ab}	570.62±16.58 ^{b}
Jiroft	626.19±6.84 ^{b}	19.97±1.45 ^{c}	472.73±5.59 ^{c}

Table 2 - Diameter of growth inhibition zone of *H. pylori* in different concentrations of different honey samples

Sample	Diameter of growth inhibition zone (mm)	
	100% solution	50% solution
Dezful	14.83±0.15 ^{c}	12.74±0.05 ^{C}
Iranshahr	13.09±0.04 ^{d}	11.32±0.07 ^{D}
Bushehr	19.17±0.12 ^{a}	17.53±0.13 ^{A}
Jahrom	18.83±0.06 ^{ab}	11.65±0.13 ^{D}
Jiroft	18.36±0.15 ^{b}	14.22±0.07 ^{B}

Table 3 – Presence of *H. pylori*, inflammation, degeneration, and eosinophil in the gastrointestinal tract of mice, according to the Sydney System classification scale (0 = Absent, + = Mild, ++ = Moderate, +++ = Severe)

Sample	Inflammation	Degeneration	Eosinophil
Dezful	1	3	1.5
Iranshahr	0.5	2	1.5
Bushehr	0.5	1	3
Jahrom			
Jiroft	1.5	1	1.5
Negative control			
Positive control			

Table 4- Correlation between antioxidant and antibacterial activities of honey samples against *H. pylori*

Antibacterial perqperties		ABTS	DPPH	FRAP
	Pearson correlation	0.929	0.851	0.672
	Sig	0.000	0.000	0.000
	N	15	15	15

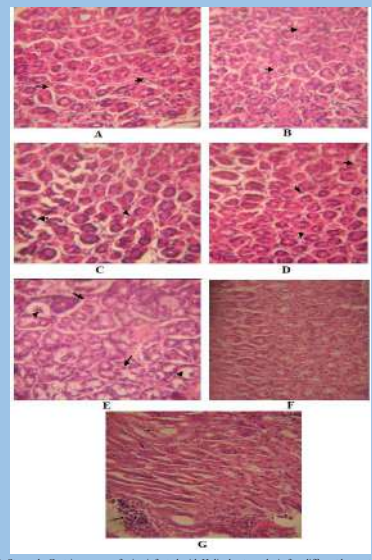

Fig4- (a-g). Stomach. Gastric mucosa of mice infected with Helicobacter pylori after different honey treatment groups
Stomach. Dezful. Mild inflammatory process and severe degeneration in the gastric mucosa were observed. Haematoxylin and eosin× 400.
Stomach. Bushehr. Mild inflammatory process, mild degeneration and severe presence of eosinophils in the gastric mucosa were observed. Haematoxylin and eosin× 400.
Stomach. Jahrom. Mild inflammatory process, relative degeneration and moderate to severe presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.
Stomach. Positive control. Moderate inflammatory process, mild degeneration was observed. Haematoxylin and eosin× 400.
Stomach. Jiroft. Mild inflammatory process, moderate to severe degeneration and severe presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.
Stomach. Negative control. Haematoxylin and eosin× 400.
Stomach. Iranshahr. Mild inflammatory process, moderate to severe degeneration and moderate presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Acknowledgement
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Tehran University

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