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Bee propolis in the treatment of *Helicobacter pylori*: in the right way to clinical application

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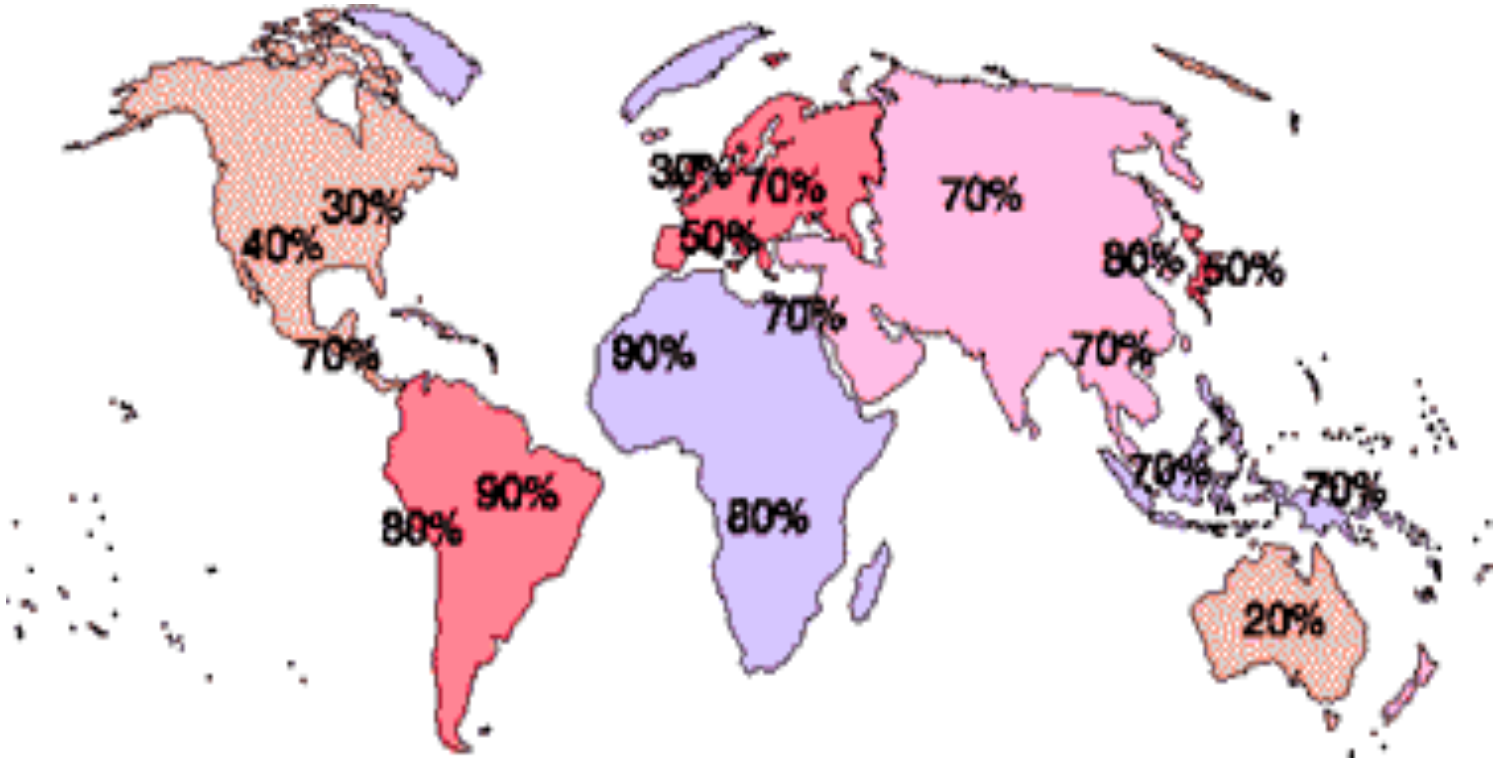
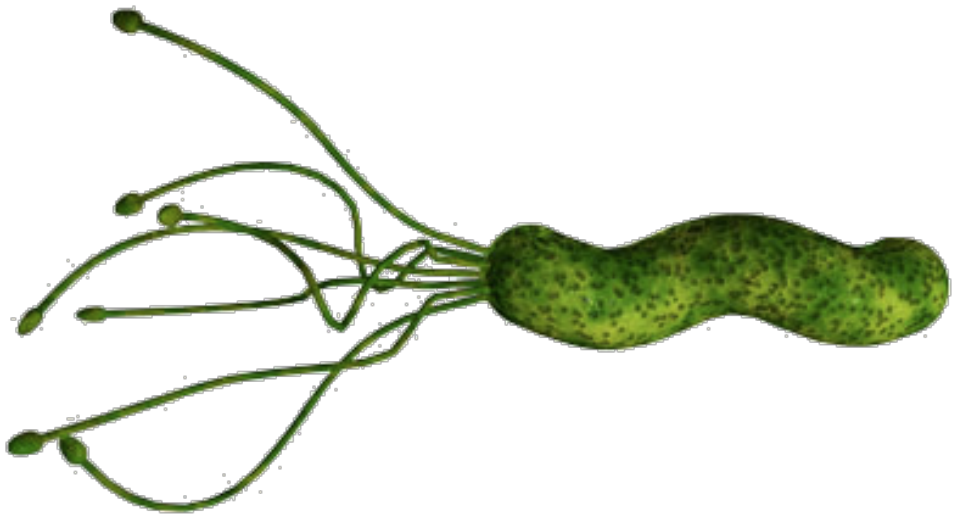
Helicobacter pylori

Helicobacter pylori infection is the most widespread infection and over than a bilion of people worldwide are affected.

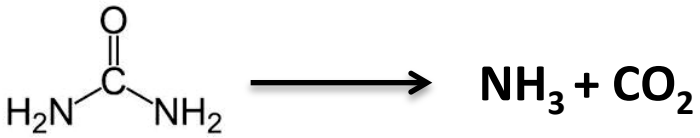


Helicobacter pylori

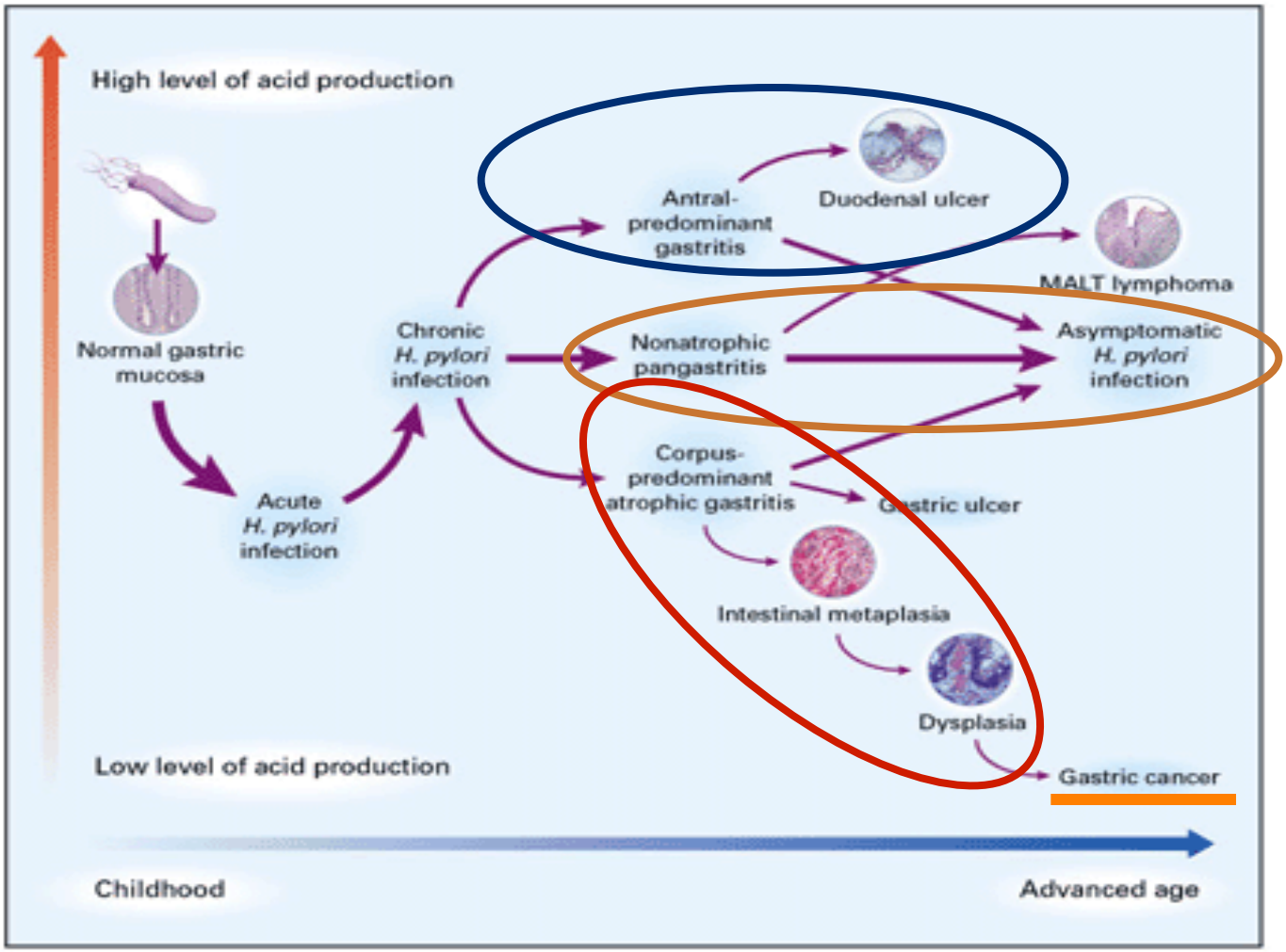


Isolated in 1982, *Helicobacter pylori* is a Gram- flagellated bacterium, able to live in the human stomach acidic and microaerophilic environment.
HP have **hydrogenases** that convert molecular hydrogen of intestinal flora in energy, **catalases, oxidases** and **ureases.**

Urease convert urea in carbon dioxide and ammonia increasing pH iand facilitating the colonization of the bacterium.



Helicobacter pylori: clinical evolution



Helicobacter pylori: pathogenesis

The most important gastric diseases related to HP infections are:

-**Gastritis**

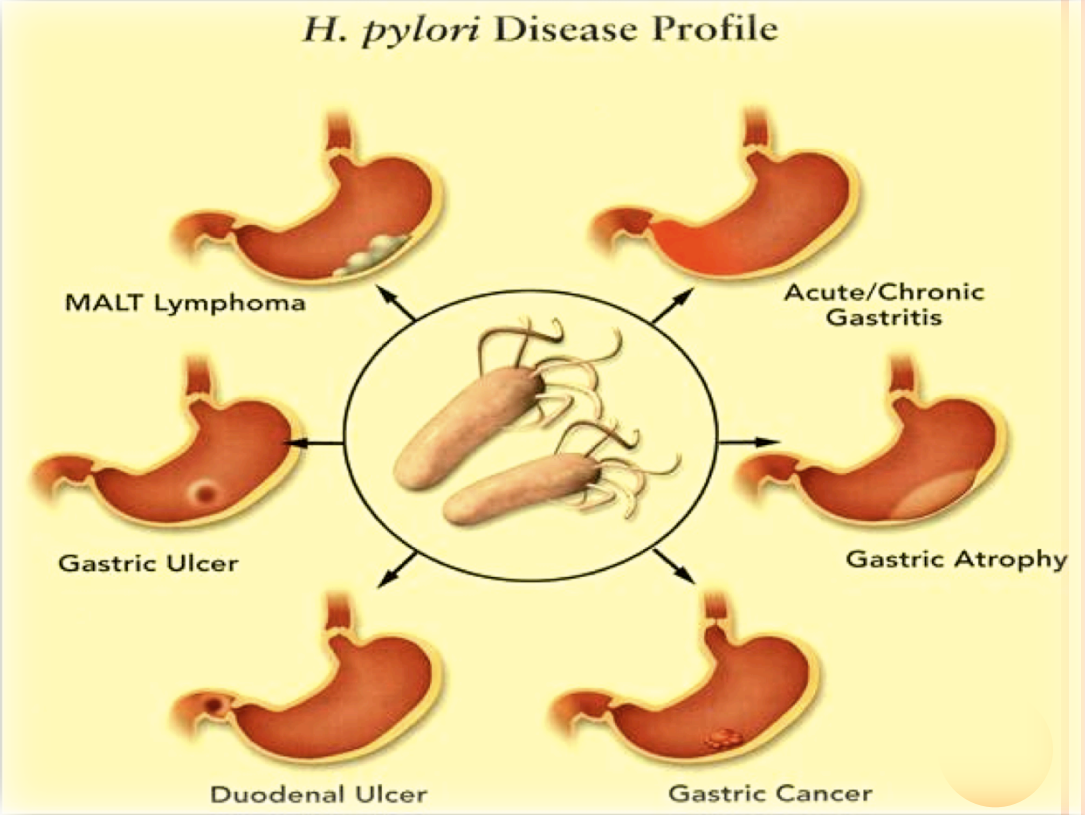
-**Peptic ulcer**

-**Metaplasia**

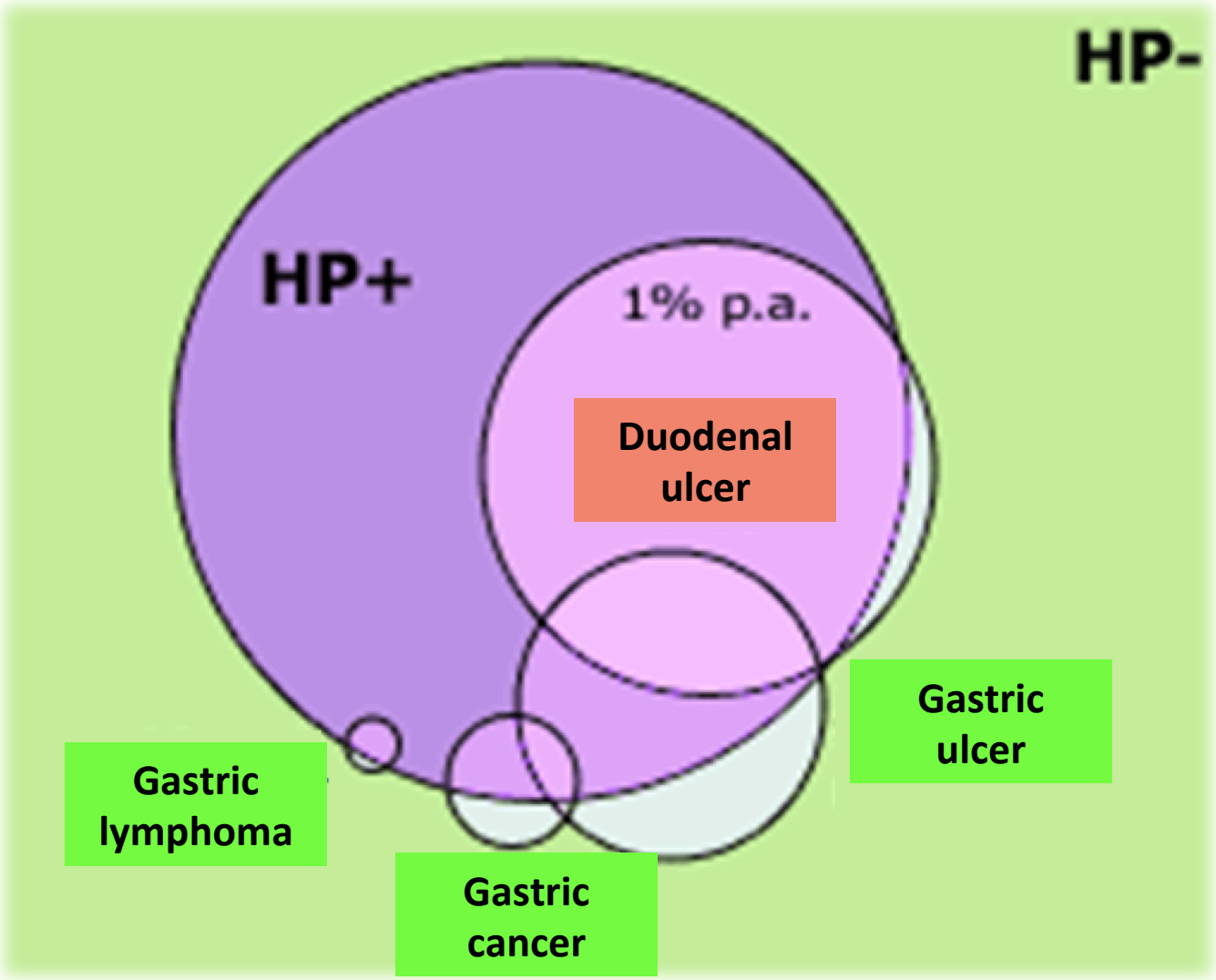
-**Dyspepsia**

-**Gastric carcinoma**

-**MALT Lymphoma**



Helicobacter pylori: pathogenesis



Helicobacter pylori: virulence factors

Pathogenicity:

- Adhesins (BabA/B, SabA): penetration and adhesion to the gastric mucosa

- Bacterial lipopolysaccharide: massive inflammatory response

-**Urease**: adhesion factor, nitrogen source, human DNA damage, increase of inflammatory response

-**HP-NAP** (HP neutrophil activating protein): *increase of ROS and cytokines release.*

- VacA protein: *vacuolizzant and cytotoxic protein. VacA alters cell tight junctions, increase* Ni^{2+}, Fe^{3+} *ions and othe r bacterial nutrient influx.*

- CagA protein: *massive inflammatory response (IL-6, IL-8, IL-18, IL-1*β *release) and macrophages activation. Alteration of gastric epithelial cells proliferation.*

Helicobacter pylori: conventional treatment

According to **Maastricht/Florence V Consensus 2016** guidelines, the conventional anti-HP treatment is:

I Line: 14 days

PPI
Clarithromycin 500 mg x 2
Amoxicillin 1 g x 2

or

PPI
Clarithromycin 500 mg x 2
Metronidazole 500 mg x 2

Failure

II Linea: 14 days

PPI
Bismuth salts 120 mg
Metronidazole 500 mg
Tetracycline 500 mg

o

PPI
Tetracycline 500 mg x 2
Metronidazole 500 mg x 2

o

PPI
Amoxicillin 1 g x 2
Metronidazole 500 mg x 2

If failure occur: Levofloxacin- containing triple or quadruple therapy may be recommended

Helicobacter pylori treatment: issues and limits

- ***Eradication rate not > 80-85%***
- **Metronidazole resistance (>40%)**
- **Clarithromycin (>20%)**

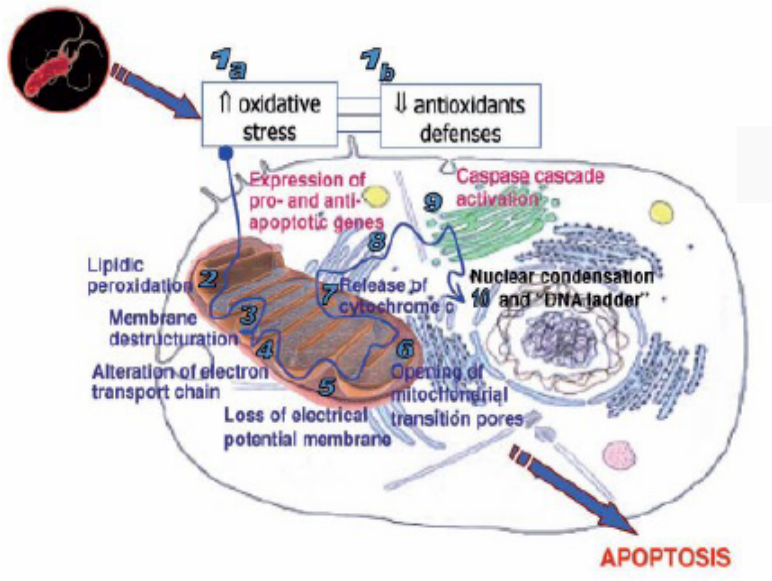
- **Side effects (gut flora damage, fatigue)**

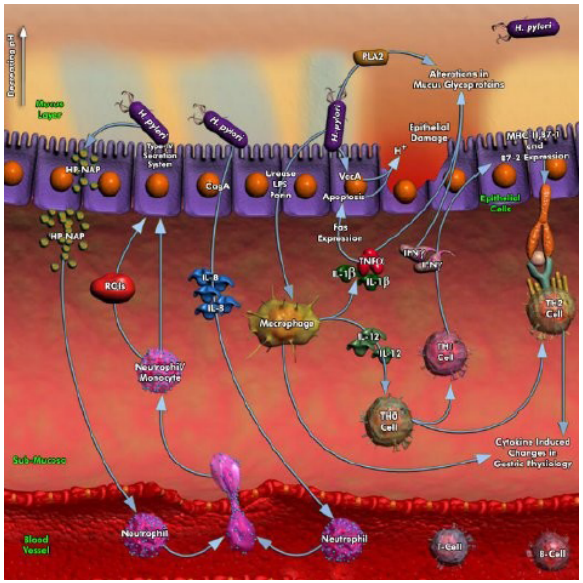
Helicobacter pylori treatment: issues and limits

HP eradication do not resolve inflammatory symptoms in the first weeks after treatment.

- > 50% of subjects with gastric ulcer mantain symptoms;

- Oxidative stress.





Propolis in the Helicobacter pylori *treatment*

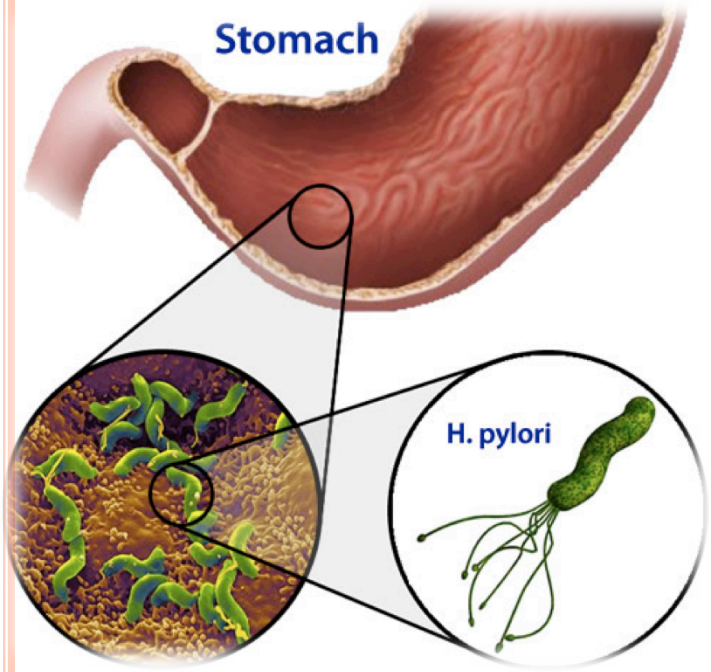
Rationale of the choice:

- Clinical investigations refer the effectiveness of propolis against *Helicobacter pylori* infections.

- Antioxidant and anti-inflammatory properties

- Safety profile

Our work



1-Comparison of different propolis marketed in Italy.

2-Evaluation of the effectiveness against several HP strains.

3-gastric stability.

4-Evaluation of the synergy between propolis and anti-HP antibiotics.

5-*in vitro* anti-inflammatory activity.

Tested samples

Propolis dried extract (12-15% total flavonoids) **(WIPE)**

Hydrodispersible propolis dried extract (8-10% total flavonoids) **(HDPE)**

Standardized 50% total polyphenols propolis based dried extract **(FEPE)**

Chemical analyses and antiradicalic and antioxidant *in vitro* essays

sample	Total polyphenols %	Flavonoids %	IC50 (DPPH) μg/ml	Ox. Pot. V
FEPE	**50,82% ± 1,66**	**11,23% ± 0,84%**	**10,2 ± 0,9**	**0,30 – 0,50**
HDPE	18,08% ± 2,64	**11,47% ± 0,11%**	10,4 ± 0,6	0,30 – 0,57
WIPE	18,95% ± 3,36	12,02% ± 0,67%	65,4 ± 1,4	0,32 – 0,52

anti *Helicobacter pylori* activity

Prop.	*MIC mg/l*	*MBC mg/l*	*HP strain*
HDPE	>2500	>2500	**10K**
FEPE	**625**	**1250**	VacA+ CagA+
WIPE	1250	2500	
HDPE	>2500	>2500	**G21**
FEPE	**625**	**1250**	VacA+ CagA-
WIPE	625	1250	
HDPE	>2500	>2500	**G27**
FEPE	**625**	**1250**	clar. resistant
WIPE	1250	2500	
HDPE	>2500	>2500	**328**
FEPE	**625**	**1250**	IC gastric carcinoma
WIPE	625	1250	
HDPE	>2500	>2500	**CCUG**
FEPE	**625**	**1250**	metron. resistant
WIPE	1250	2500	

MIC: minimum inhibitory concentration

MBC: minimum bactericidal concentration

Influence of bacterial concentration on FEPE propolis extract activity

Strain :
CCUG metron. Res.

Sample:
FEPE

CFU/ml	MIC mg/l	MBC mg/l
10^3	625	1250
10^6	625	1250
10^9	625	1250

No MIC or MBC variations .

Killing Time

Strain :
CCUG metron. Res.

Sample:
FEPE

Time	MBC mg/l
2 h	--
4 h	1250
24 h	1250

Propolis is effective against HP in 4 h.

The mechanism of action is probably related to a physical bacterial wall damage.

≠ biochemical mechanism of antibiotics

Gastric stability of propolis extract

According to USP e McDougall 2006 (modified)

pH	T_0 polyphenols mg/l	post digestion polyphenols mg/l	recovery
1,7	418,48 ± 2,81	384,76 ± 24,95	**91,20% ± 5,86%**
4,5	418,48 ± 2,81	377,93 ± 25,50	**89,36% ± 5,99%**

Antibacterial synergy between propolis and antibiotics

Antibiotics:

Clarithromycin, 1 mg/l

Metronidazole, 64 mg/l

Tetracycline, 8 mg/l

Levofloxacin, 16 mg/l

Amoxicillin, 2 mg/l

Strains:

1-CCUG, metronidazole resistant

2- G27, clarithromycin resistant

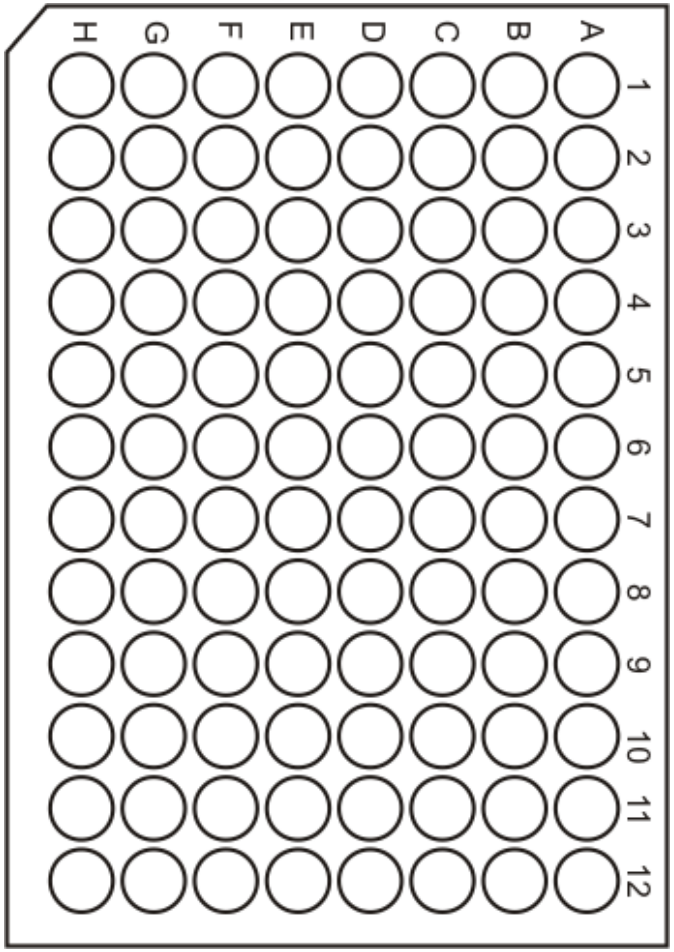
Propolis extract:

FEPE

Checkerboard method

200 µL FEPE in BB

100 µL antib.



Evaluation of FIC and FBC indexes according to EUCAST

Antibacterial synergy between propolis and antibiotics

Propolis and Clarithromicin

CCUG metron.res.	FIC	FBC	FIC index	FBC index
Propolis	n.d	n.d	n.d	n.d
Clarithromycin	<0,250	<0,250		
IC clarit. res.	**FIC**	**FBC**	**FIC index**	**FBC index**
Propolis	0,250	0,250	0,500	0,500
Clarithromycin	0,250	0,250		

Antibacterial synergy between propolis and antibiotics

Propolis and Metronidazole

CCUG metron.res.	FIC	FBC	FIC index	FBC index
Propolis	0,250	0,500		
Metronidazole	0,250	0,031	0,500	0,531
IC clarit. res.	**FIC**	**FBC**	**FIC index**	**FBC index**
Propolis	0,500	0,500		
Metronidazole	0,250	0,250	0,750	0,750

Antibacterial synergy between propolis and antibiotics

Propolis and Tetracycline

CCUG metron.res.	FIC	FBC	FIC index	FBC index
Propolis	0,500	1,000		
Tetracycline	0,125	0,031	0,625	1,031
IC clarit. res.	**FIC**	**FBC**	**FIC index**	**FBC index**
Propolis	0,500	0,500		
Tetracycline	0,016	0,016	0,516	0,516

Antibacterial synergy between propolis and antibiotics

Propolis and Levofloxacin

CCUG metron.res.	FIC	FBC	FIC index	FBC index
Propolis	0,250	0,125		
Levofloxacin	0,125	0,063	0,375	0,188
IC clarit. res.	**FIC**	**FBC**	**FIC index**	**FBC index**
Propolis	1,000	1,000		
Levofloxacin	0,500	0,500	1,500	1,500

Antibacterial synergy between propolis and antibiotics

Propolis and Amoxicillin

CCUG metron.res.	FIC	FBC	FIC index	FBC index
Propolis	0,250	0,250		
Amoxicillin	≤0,016	0,008	<0,266	0,258
IC clarit. res.	**FIC**	**FBC**	**FIC index**	**FBC index**
Propolis	0,250	0,250		
Amoxicillin	0,250	0,250	0,500	0,500

Antibacterial synergy between propolis and antibiotics

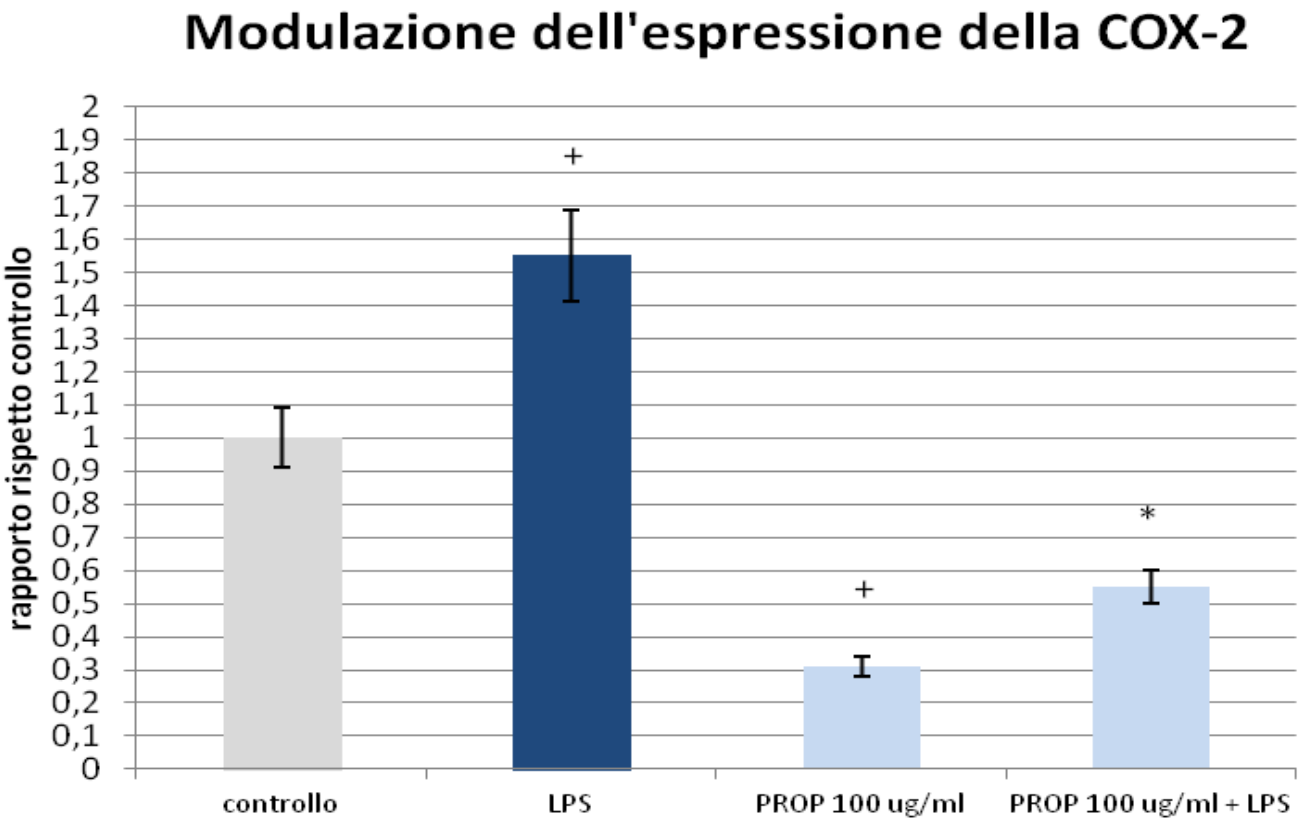
-**Bacteriostatic *synergy*** (FIC indexes) occur in **60% of tested situations**;

-***Bactericidal synergy*** (FBC indexes) occur in **50% of cases.**

In 30% of cases FICi and FBCi show that propolis and antibiotics effects are additive.

In worst cases propolis and antibiotics have indifferent synergy.

ex vivo PBMC anti-inflammatory activity



Discussion

Our findings showed that propolis could be used conveniently in clinical practices in HP infections management, in particular in association with conventional PPI-antibiotic therapy.

1- HP loss of resistance

2- Synergistic effect of prpolis and antibiotics

3- Antioxidant and anti-inflammatory effectiveness

Propolis integration in the HP treatment could increase eradication rate, help in HP related symptoms, decrease side effects and have positive ripercussions on patients' compliance.