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In Vivo Study of Probiotic Role in Protection and Prevention of *Helicobacter Pylori* that Cause Stomach Ulcer in Rats

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Abstract:
To evaluate the probiotic role in protection and prevention of Helicobacter pylori that cause stomach ulcer. *H. pylori* were isolated from patients with gastric ulcers. The probiotic was purchased from pharmacy which used in this study. Forty male rats were divided into 4 groups, G1 serve as control negative given only distil water, G2 given only Probiotic for 30 consecutive days, G3 given only *H. pylori* at 10^7 CFU/ml, G4 given Probiotic for 30 consecutive days then infected with *H. pylori* at 10^7 CFU. The results of invitro study showed susceptibility of H. pylori isolates to Probiotic and Ciprofloxacin, while it presented resistant to other antibiotics used. The histopathological results showed the bacteria appeared to be adherent to the gastric epithelium in G3. The rats developed chronic active gastritis in G3 closely resembling the *H. pylori* gastritis observed in humans, while the histopathological G4 showed apparently normal mucosa.

In conclusion, the usage of probiotics for 1 month can decrease the chance of *H. pylori* infection that causing gastric ulcer.

Keywords: probiotic, *H. pylori*, rats

Introduction:

The primary source of recurrent acute gastritis, peptic ulcers, gastric lymphoid tissue lymphoma combined with mucus and gastric cancer is *Helicobacter pylori (HP).* Dyspepsia, anemia and idiopathic thrombocytopenic purpure are also strongly correlated with it (Malfertheiner *et al*., 2012).

Colonization of human stomachs in about 70% of the population by Helicobacter pylori is asymptomatic. While *H. Pylori* is colonized by approximately half the citizens. *H. Pylori* are expected to develop peptic ulcer only around 10–20%, whereas gastric or mutation-associated lymphoma of the lymphoid tissue are likely to develop about 1–2%. (Marshall, 2006).

There is actually no rehabilitation scheme for 100% HP eradication. The rate of eradication is correlated with various variables like treatment, patient tolerance for adverse reactions, patient conformity, patient genetic polymorphism, obesity, diabetes and other factors. (Lin *et al*., 2017). Among these, the major cause of failure to treat HP eradication is antibiotic resistance to HP (Graham *et al*., 2014).

Several research in animals and humans affirm the good health and well-being impact of probiotics in people with dysbiosis conditions (Williamson *et al*. 2017; Finley *et al*. 2018). For humans, convincing proofs are accumulating to help gastrointestinal dysfunction and depressive symptoms through probiotics (Nadeem *et al*. 2019). In the other instances of dysbiosis-related diseases, including obesity, cardiovascular disease, diabetes, and cancer, a growing level of focus has also been given to probiotic functions (Mazloom *et al*. 2019; Tang *et al*. 2019).

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Incompatible test reports have nevertheless avoided assumptions concerning HP's therapeutic prowess. Mechanisms for probiotic acts require the development, for the adherence to gaseous epithelial cells, of substances that inhibit or destroy HP or fight for HP. Probiotics may also minimize infection by controlling a local host's immune response (Song *et al*., 2018).

The aim of this study was to evaluate the probiotic role in protection and prevention of Helicobacter pylori that cause stomach ulcer.

Materials and Methods

H. pylori was isolated from patients with gastric ulcers and stored at −80°C (Markey *et al*., 2013). The probiotic was purchased from pharmacy which used in this study.

Forty male rats were divided into 4 groups as follows:

G1 serve as control negative given only distil water.

G2 given only Probiotic for 30 consecutive days

G3 given only *H. pylori* at 10^7 CFU/ml (Sgouras *et al*., 2004).

G4 given Probiotic for 30 consecutive days then infected with *H. pylori* at 10^7 CFU

The probiotic was observed by invitro study according to (Hudzicki, 2009). Also, five antibiotics were used for invitro study (Ciprofloxacin, gentamicin, Amoxicillin, Ceftriaxon and Sulfa), these were purchased from Bio-Rad company (Hudzicki, 2009).

Results:

The results of invitro study showed susceptibility of *H. pylori* isolates to Probiotic and Ciprofloxacin, while it presented resistant to other antibiotics used (Fig. 1,2).

Table 1: Zone of inhibition for probiotic, Ciprofloxacin, gentamicin, Amoxicillin, Ceftriaxon and Sulfa on *H. pylori* in vitro.

Discs	Zone of inhibition (diameter/ mm)	Sensitivity	Resistant
Probiotic	28	✓	
Ciprofloxacin	31	✓	
Gentamicin	13		✓
Amoxicillin	0		✓
sulfa	9		✓
Ceftriaxone	8		✓

($P<0.05$)

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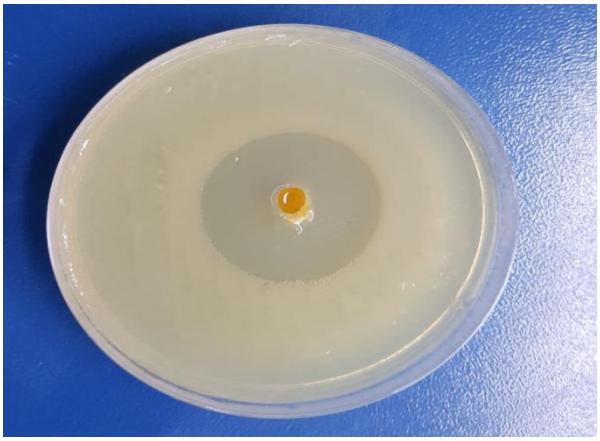

Fig. 1. Invitro usage of probiotics against *H. pylori*

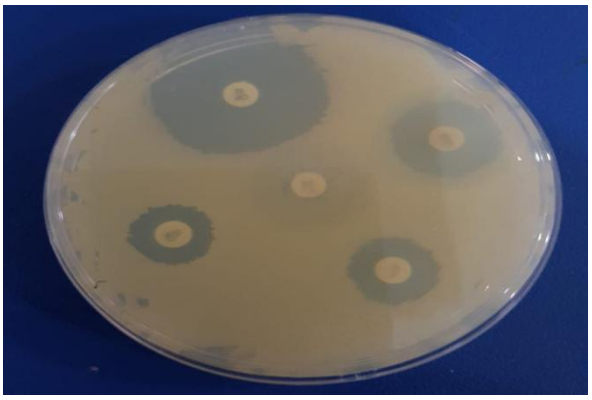

Fig. 1. Invitro usage of different antibiotics against *H. pylori*

H. pylori was noticed in the gastric samples by a viable count. Histopathologic assessment exposed the existence of *H. pylori* in all rats in G3.

The existence of bacterial *H. pylori* and linked gastritis wasn't detected in any of the uninfected control animals. the bacteria looked to be adhere to the epithelium of stomach of G3 (Fig. 1). The rats established a chronic active gastritis in G3 (Fig.2) which closely like the gastritis caused by *H. pylori* that detected in humans, while the Fig. 3 showed apparently normal mucosa in G4.

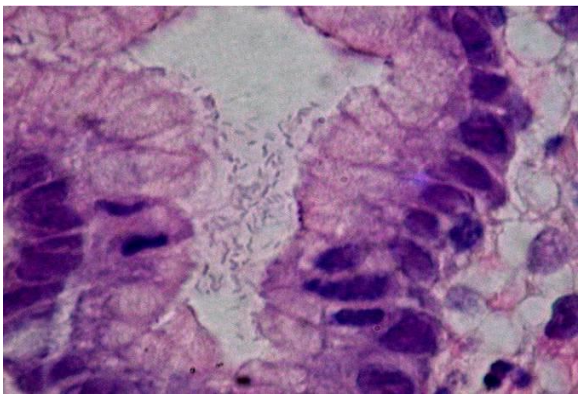

Fig. 1. Histophotography of gastric mucosa showed Note the presence of *H. pylori* in the gastric pits in G3, H&E x40

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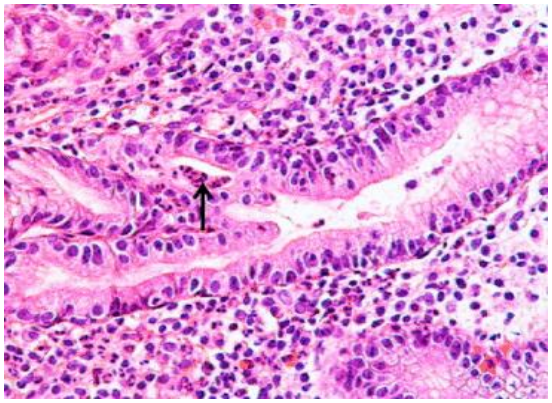

Fig.2. Histophotography of gastric mucosa showed the presence of chronic active inflammation in the lamina propria G3, H&E x40

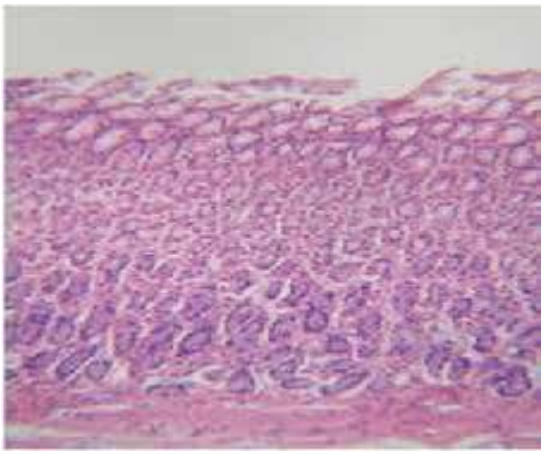

Fig. 3. Histophotography of gastric mucosa showed apparently normal mucosa in G4, H&E x40

Discussions:

Probiotics are characterized as live micro-organisms that provide the host with a health benefit when properly administered (Dore *et al*., 2019).

A variety of gastrointestinal disorders, including *H pylori*, were treatment by probiotics as an emerging adjuvant. In vitro studies have indicated the likelihood of antagonizing in different probiotics (Chen *et al*., 2019). Zhao *et al*. (2018) found 4 strains of Lactobacillus that isolated from the fermented foods in China were capable to prevent the multiplication of bacteria *H. pylori*.

A variety of studies indicate that probiotics not only avoid the growth of acute gastric mucosal lesions, but also speed up the healing phase of caused gastric ulcers (Uchida *et al*., 2010; Singh and Kaur, 2012).

Probiotics can secrete antibacterials such as lactic acids, SCFAs, hydrogen peroxides and bacteriocin which act as antibacterial (Homan and Orel, 2015). Lactic acid and SCFAs are typically more severe than strong antibacterial acids because of incomplete ionization, and *Helicobacter pylori* cells are damaging by acting as a proton carriers that trigger cytoplasm acidification and aggregation of toxic anions (Poppi *et al*., 2015). Zheng *et al*. (2016) done an in vitro research was performed by using Lactobacilli against *H. pylori*, which showed that both drug-sensitive and drug-resistant can be blocked by lactic acid. Also, it has been

reported that lactic acid can suppress the urease activity of *H. pylori* (Lesbros-Pantoflickova *et al*., 2007). In addition to organic acids, probiotic-producing hydrogen peroxide that harm for bacteria, membrane lipids and DNA oxidative damage by forming peroxygen ions., all hurting the cell of *H. pylori* (Batdorj *et al*., 2007). In addition, some probiotics can generate bacteriocins which directly affect on cells of *H. pylori*. Some bacteriocins are thermostable, planktonic cells or biofilm cells antagonistic peptides (Kim *et al*., 2019).

Conclusion:

Probiotics can eradicate and prevent the *H. pylori* infection that causing gastric ulcer.

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