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EFFECT OF BIFIDOBACTERIUM BIFIDUM CUETM 89/29 ON HELICOBACTER PYLORI 158 SAN RESPONSIBLE FOR GASTRODUODENAL DISEASES

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ABSTRACT

Helicobacter pylori is a pathogenic bacterium recently recognized for its implications in gastroduodenal diseases; duodenal ulcer, gastric ulcer, gastric lymphoma of MALT and gastric cancer. Lactic acid bacteria, such as Bifidobacteria play an important role in nutrition, for treatment and prevention of diseases in medicine and for conservation of commercial food products due to their interesting characteristics and antimicrobial properties.

The objective of our work is to demonstrate the in vivo probiotic effect of *Bifidobacterium bifidum* CUETM 89/29 on *Helicobacter pylori* SAN 158. Histological sections of rabbit gastric biopsy indicate that the feeding of *B. bifidum* has a significant impact on inflammation caused by *H. pylori.* These results confirmed that *B. bifidum* CUETM 89/29 inhibits *H. pylori* SAN 158.

This study suggests that lactic acid bacteria are a good candidate used to prevent human enteric infection.

KEYWORDS: *Helicobacter pylori*, Gastrointestinal Diseases, Probiotic Effect, *Bifidobacterium bifidum*

INTRODUCTION

The discovery of the role of *Helicobacter pylori* in some gastroduodenal diseases came upset pathophysiological concepts **(Fagniez, 1995, Mégraud, 1996).** *Helicobacter pylori* is involved in the genesis of the majority of chronic gastritis, ulcer gastric or duodenal diseases, non-ulcer dyspepsia or indirectly in the development of lymphoma and gastric cancer **(Ferrero *et al.*, 1995).** This is certainly an excellent marker for these diseases **(Fauchèr and Rosenau, 1991)**.

Bifidobacteria are the interest of doctors and nutritionists since their discovery in 1899 and 1900 by Tisser because of their absence in pathogenicity and reputation of protection against gastroenteritis. These bacteria produce lactic acid and acetic acid, which lowers the pH and subsequently inhibit the growth of certain pathogens. This inhibition is also due to the bacteriocins and the production of H_2O_2 by Bifidobacteria **(Rasić and Kurmann, 1983**).

In this work, we have integrated *H. pylori* with intestinal Bifidobacteria to see at what degree *B. bifidum* inhibits the growth of this pathogen?

MATERIAL AND METHODS

Materials

Test Organism

- *Helicobacter pylori* was isolated and identified in the laboratory of bacteriology of Pellegrin Hospital - Bordeaux (France).

- *Bifidobacterium bifidum* CUETM 89/29 is a reference strain which is obtained from INRA Institute - Rennes (France)**.**

Laboratory Animals

The experiment was carried out on rabbits of the same age (1-3 months) and weighing between 400 and 990 g. These rabbits were individually placed in well ventilated metal cages and kept in the animal house of Veterinary Sciences Institute - University of Ibn Khaldoun – Tiaret (Algeria).

METHODS

The experimental protocol is summarized in the following diagram:

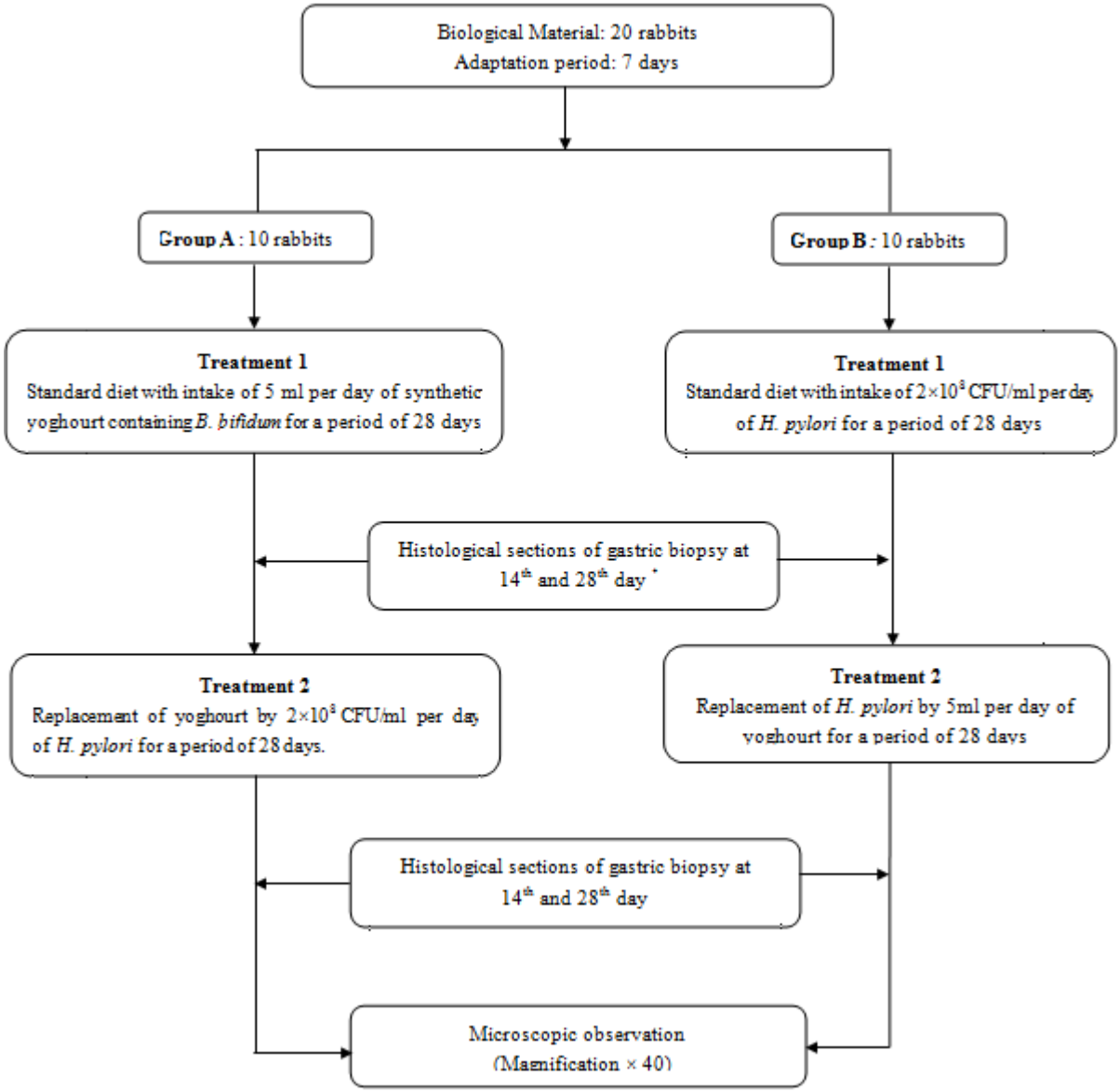

Figure 1: Experimental Protocol of *In Vivo* Study

* Procedures of dissection and histological sections were realized according to **Jean Pierre (2007).**

RESULTS

Group A

- **Treatment 1**

The histological structure of rabbit stomach on the 14th day of *B. bifidum* intake was complete and well-developed **(Figure 2).** Nevertheless, at 28th day of treatment, an increase in lymphoid components was observed. Peyer's patches were very voluminous **(Figure 3).**

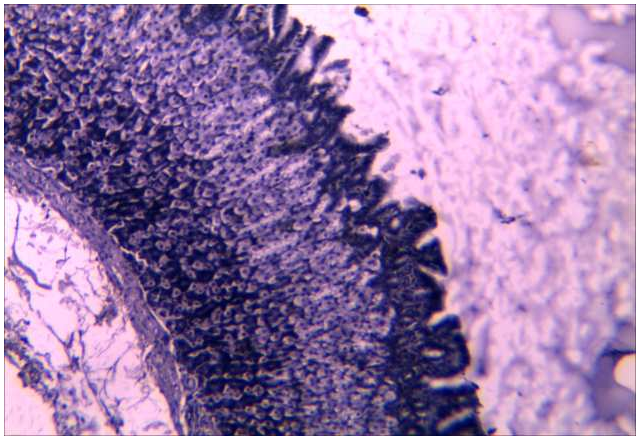

Figure 2: Histological Section of the Stomach (After 14 Days of *B.bifidum* Intake)

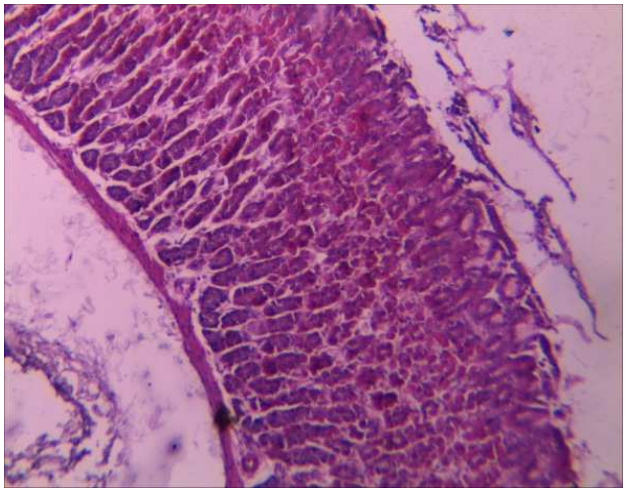

Figure 3: Histological Section of the Stomach (After 28 Days of *B.bifidum* Intake)

- **Treatment 2**

After 14 days of *H. pylori* intake, rabbits were not eating well and physical activity has decreased with weight drop abdominal swelling and early presence of diarrhea**.** At the end of the 28 th day of *H. pylori* intake, stomach swelling was observed with bad odor during dissection.

Histological sections of stomach rabbit after 14 days of *H. pylori* intake show a beginning of inflammation with a very low degree. Some of villi have become atrophied and high number of white blood cells were occurring as immune response **(Figure 4).** But after 28 days of treatment, the infection was very clear with the disappearance of some villi and opening to their ends which indicating the beginning of their destruction. Also, Liberkuhn glands appear as invaginations of the epithelium during this period **(Figure 5).**

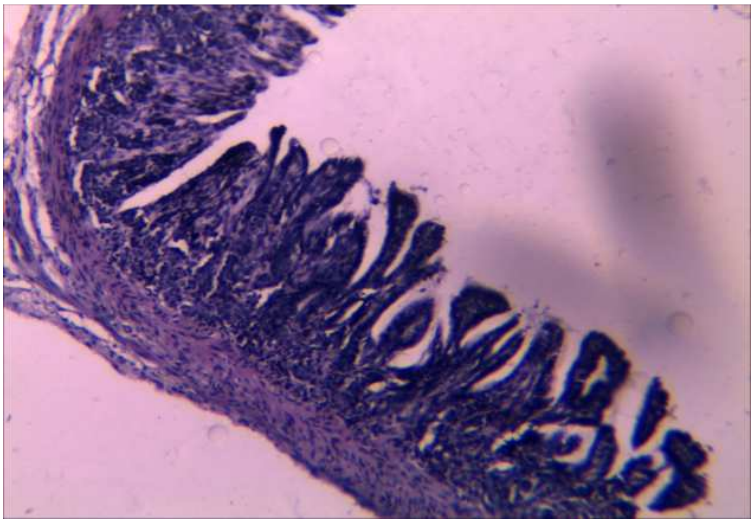

Figure 4: Histological Section of the Stomach (After 14 Days of *H.pylori* Intake)

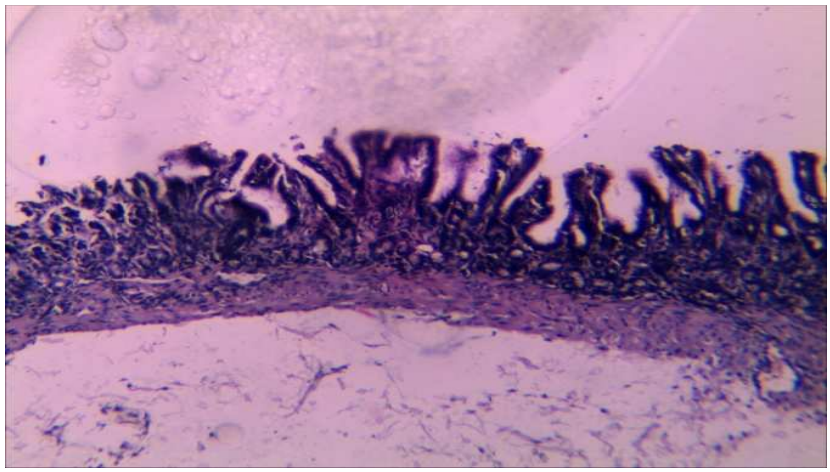

Figure 5: Histological Section of the Stomach (After 28 Days of *H.pylori* Intake)

Group B

- **Treatment 1**

During the first 14 days, the rabbits were in poor physical condition with weight drop, abdominal swelling and presence of diarrhea**.** At the end of the 28 th day, the inflammation was aggravated with a very bad odor during dissection and irritation of the stomach and intestine. During this period, rabbit's death was observed.

Histologically, the inflammation is well developed. Villi, lymphoid nodules and some epithelial layers were substantially disappeared with increase of white blood cells number after 14 days of *H.pylori* intake **(Figure 6).** At the end of the 28th day of treatment, complete disappearance of villi is observed and some of muscularis mucosae and lymphoid nodules became smaller **(Figure 7).**

Figure 6: Histological Section of the Stomach (After 14 Days of *H.pylori* Intake)

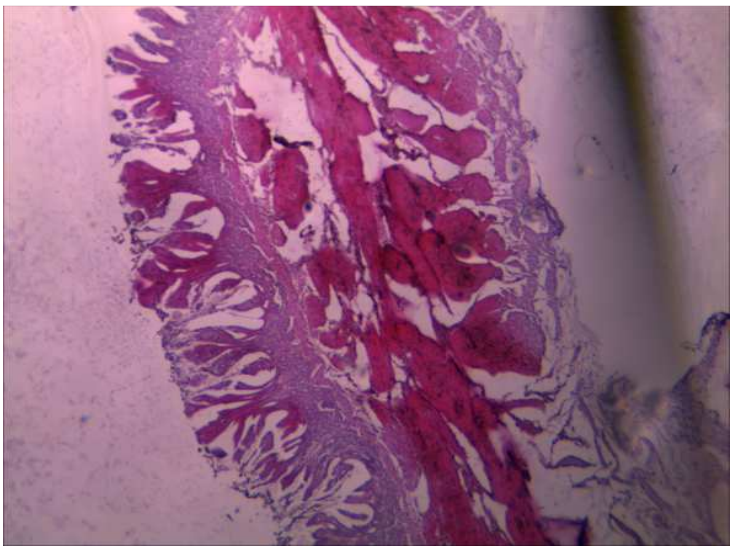

Figure 7: Histological Section of the Stomach (After 28 Days of *H.pylori* Intake)

- **Treatment 2**

After 14 days of *B. bifidum* intake, the rabbits still suffering from diarrhea and abdominal swelling gradually disappears. They regained their normal physical conditions with cure beginning after 28 days of treatment.

The inflammation persists and the villi and some layers (mucosa and submucosa) were almost unfounded after 14 days of *B. bifidum* intake **(Figure 8).**

The Inflammation progresses toward healing with restoration of some layers (mucosa and muscularis mucosae) after 28 days of *B. bifidum* intake .Lymphoid nodules join their shape and are distributed in an organized manner at muscularis mucosae **(Figure 9)**.

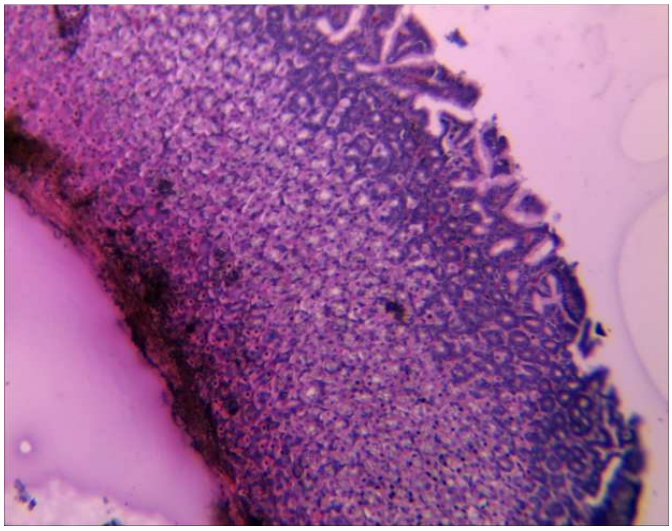

Figure 8: Histological Section of the Stomach (After 14 Days of *B.bifidum* Intake)

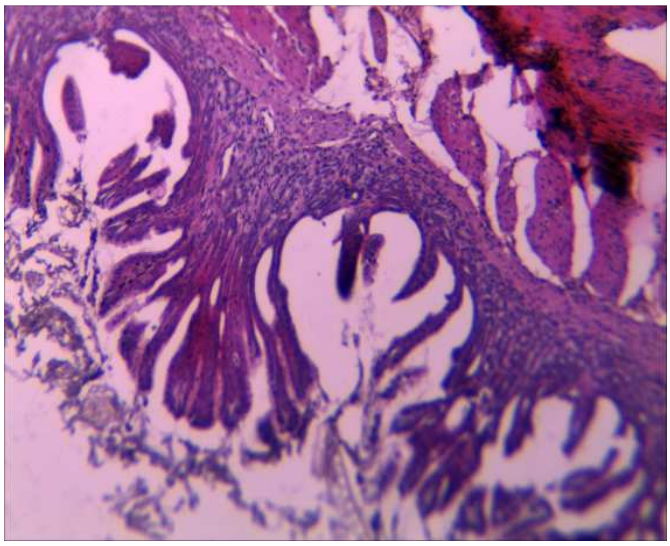

Figure 9: Histological Section of the Stomach (After 28 Days of *B.bifidum* Intake)

DISCUSSIONS

Recent animal studies have indicated that some commercial probiotic strains can increase the resistance against colonization and infection by enteric bacteria **(Bouhnik *et al.*, 1996).**

This study aims to test the probiotic effect of *B. bifidum* alimentation on gastroduodenal infections caused by *H. pylori*.

The results of the first treatment given to Group A (feeding by *B. bifidum)* show a significant impact on inflammation caused by *H. pylori* because the digestive tract in this case is considered immunized. The rabbits of Group B which take *H. pylori* as first treatment remain more susceptible to suffering from gastric illness.

The histological results indicate that replacement of *H. pylori* by *B. bifidum* in the second treatment generates an immune response with the healing of damaged tissues. In our case, the inflammation is not completely cured; this is due to the shortness of treatment duration.

Many mechanisms have been postulated by which probiotics may inhibit enteric pathogens ; the removal of intestinal pathogens by lactic acid bacteria has been attributed to their ability to alter the intestinal microenvironment by secreting organic acids, mainly acetic acid and lactic acid, leading to acidic pH and by producing antibacterial compounds **(Servin, 2004) . Tabak *et al* (2007)** showed that the high concentration of organic acids at low pH in *B. bifidum* that colonizes the intestine inhibits *H. pylori* toxin production while **Fuller (1989)** have shown that Bifidobacteria produce a protein factor that inhibits the adhesion of certain *H. pylori* strains to GAI *in vitro*.

Probiotics produce organic acids and antimicrobial molecules, and develop many mechanisms which can be involved in the protection of the digestive tube against the gastroduodenal pathologies **(Gill, 2003, Tabak *et al.*, 2012).** And according to **Saavedra *et al* (1994),** Bifidobacteria increase the number of caliciform cells, which are an intestinal epithelial response to inflammation, and secrete a viscous gel that trap and limit the access of microorganisms to the epithelium.

Desmazeaud (1983) showed that the effects of the proliferation of certain pathogenic strains may be inhibited by the ingestion of lactic ferments by the following mechanisms:

- Secretion of certain antimicrobial substances by Bifidobacteria (H_2O_2, lactic acid, acetic acid).
- Lowering the pH by acidic products.
- Prevention of toxic amine synthesis.
- Barrier effect of metabolic competition or preventing pathogen colonization by binding to the gastrointestinal tract.
- Degradation of enterotoxin by detoxification.

Certain strains of Bifidobacteria play a protective role against the necrotic neonatal enterocolitis (NEC), avoiding the development of the latter by inhibition of their growth as *Clostridium* **(Bouhnik *et al.*, 1996).**

Carmen *et al* (2000) confirmed the presence of bacteriocin produced by *B. bifidum* inhibiting *H. pylori. B. bifidum* strain can naturally produce more than a bacteriocin **(De Vuyst and Vandamme, 1994).** These substances were characterized as proteinaceous molecules **(Delves Broughton, 1990)** and are therefore sensitive to the action of different proteolytic enzymes.

CONCLUSIONS

The obtained results showed that the intake of probiotics in the diet of rabbits generates a protection against infection by *Helicobacter pylori*. This study suggests that probiotics are a good candidate used for prevention against gastroduodenal infections. Today, *Bifidobacterium* occupies a vast area for carrying out numerous taxonomic, ecological and medical studies. Future directions of *Bifidobacterium* bacteria in the food and medical fields are certainly making a better knowledge of these bacteria.

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