**ABSTRACT**

Background: *Helicobacter pylori* infection affects nearly 50% of the world's population, and 1 to 3% of patients infected with *H. pylori* have a risk of developing gastric cancer. Probiotics can inhibit the proliferation and adhesion of *H. pylori*. This study aimed to test *Lactobacillus plantarum* as an adjuvant therapy in treating *H. pylori*. Methods: Dyspeptic patients (18-74 years) with gastric infection documented and untreated were randomized into two groups: probiotic group (amoxicillin and clarithromycin for one week, esomeprazole for three weeks and *Lactobacillus plantarum* 5 x 10^8 CFU for four weeks) and placebo group (amoxicillin, clarithromycin, and esomeprazole for the same period). Four weeks after therapy, a re-endoscopy was performed. Results: Fifty patients were randomized, and 47 (mean 42.7 SD 14.8 years) concluded the study. The total eradication rate was 78.7% (p = 0.73). Further analyses by age group showed that in patients up to 45 years, the eradication rate in patients who received probiotics was 93.8% against 70.0% in the placebo group (p = 0.046). Conclusion: The results indicate the potential of using probiotics as an adjuvant in eliminating *H. pylori*, and the greater potential of probiotics use in younger age groups.

**Keywords:** Helicobacter pylori; Probiotics; Lactobacillus plantarum
RESUMO

Introdução: A infecção por *Helicobacter pylori* afeta cerca de 50% da população mundial e 1 a 3% dela apresenta risco de desenvolver câncer gástrico. Os probióticos inibem a proliferação e adesão do *H. pylori*. Este estudo objetivou testar *Lactobacillus plantarum* como terapia adjuvante no tratamento do *H. pylori*. Métodos: Pacientes dispépticos (18-74 anos) com infecção gástrica documentada e não tratada foram randomizados em 2 grupos: grupo probiótico (amoxicilina e claritromicina por uma semana, esomeprazol por 3 semanas e *L. plantarum* 5 x 10⁸ UFC por quatro semanas) e grupo placebo (amoxicilina, claritromicina e esomeprazol no mesmo período). Quatro semanas após a terapia, foi realizada uma nova endoscopia. Resultados: 50 pacientes foram randomizados e 47 (média 42,7 DP 14,8 anos) concluíram o estudo. A taxa de erradicação total foi de 78,7% (p = 0,73). Análises por faixa etária mostraram que em pacientes de até 45 anos, a taxa de erradicação em pacientes que receberam probiótico foi de 93,8% contra 70,0% no grupo placebo (p = 0,046). Conclusão: Os resultados indicam o potencial do uso de probióticos como adjuvante na eliminação do *H. pylori* e o maior potencial do uso de probióticos em faixas etárias mais jovens.

Palavras-chave: *Helicobacter pylori*; Probioticos; *Lactobacillus plantarum*

INTRODUCTION

*Helicobacter pylori* infection affects approximately 50% of the world's population (O’CONNOR et al., 2016). About 20% of infected individuals develop symptomatic chronic active gastritis, gastric or duodenal ulcer, gastric adenocarcinoma, or gastric non-Hodgkin’s lymphoma (PARSONNET et al., 1994). People infected with *H. pylori* have a 1 to 3% risk of developing gastric cancer (BERNARDINI et al., 2017). Recent studies have shown that eradicating *H. pylori* in infected asymptomatic individuals of all ages can reduce the occurrence of gastric cancer (YU et al., 2023; KAMAREHEI et al., 2023). Antibiotic therapy is the most effective and widely used treatment for *H. pylori* infection, in which clarithromycin, amoxicillin, or metronidazole can inhibit *H. pylori* infection (GODERSKA et al., 2018). However, it is believed that the triple regimen of antibiotic therapy may fail in 10% to 23% of patients (KUSTERS et al. 2006). To improve the eradication rate and decrease the adverse events of antibiotic therapy, several authors have proposed the use of probiotics as an adjuvant therapy to the classic triple regimen (ISOLAURI et al., 2001; SAVILAHTI et al., 2009; RASK et al., 2012; HOMAN 2015; SARACINO et al., 2020). Probiotics, such as lactobacilli, can inhibit *H. pylori* through various immunological and non-immunological mechanisms (BRUNO et al., 2018). In general, probiotics can inhibit the proliferation of bacteria by competing with *H. pylori* for host surface receptors and subsequently disrupt their adhesion to epithelial cells (MUKAI et al., 2002). In addition, probiotics can neutralize
spiral bacteria by secreting some antibacterial compounds, including lactic acid, bacteriocins, hydrogen peroxide, fatty acids, and antibiotic-like substances such as reuterin and reutericline (FOLIGNE et al., 2007).

Animal trials revealed that *L. plantarum* ZJ316 had preventive and therapeutic effects on *H. pylori*-induced gastritis. *L. plantarum* ZJ316 significantly decreased interferon γ (IFN-γ) and interleukin 6 (IL-6) levels, increased IL-10 levels (FORTES et al., 2020) and repaired mucosal damage. Furthermore, sequencing of the 16S rRNA gene revealed that the relative abundance of *H. pylori* could be significantly reduced by administering *L. plantarum* ZJ316. *L. plantarum* ZJ316 survived well in simulated gastrointestinal conditions and showed anti-*H. pylori* ability. Results indicate that *L. plantarum* ZJ316 is a potential candidate for preventing and treating *H. pylori*-induced gastritis by regulating the gastric microbiota and reducing mucosal inflammation (QINGGINGi et al., 2021). A recent study investigated the anti-*H. pylori* from a freshly isolated strain of *L. plantarum* (pH3A), monolaurin, and grapefruit seed extract (GSE) and their synergies *in vitro* and *in vivo*. Monolaurin and GSE suppressed *H. pylori* growth and urease activity at a minimum inhibitory concentration (MIC) of 62.5 ppm. Live cells and cell-free culture supernatant (CFCS) of *L. plantarum* pH3A with or without pH adjustment also significantly inhibited *H. pylori* growth. Although no synergy was observed between monolaurin and GSE, adding CFCS significantly enhanced its anti-*H. pylori* (SINI et al., 2021).

An experimental study carried out in rats showed that *L. plantarum* ZJ316 is a potential candidate for preventing and treating *H. pylori*-induced gastritis, regulating the gastric microbiota and reducing mucosal inflammation (ZHOU et al. 2021). Pan et al. demonstrated that pre-treatment with *L. plantarum* ZDY 2013 played an essential role in preventing inflammation of the gastric mucosa and alteration of the gastric microbiota induced by *H. pylori* infection (PAN et al. 2016). A recent prospective, randomized, placebo-controlled study showed that adding probiotics to the concomitant 10-day bismuth-free regimen for *H. pylori* eradication increases the eradication rate and decreases side effects. The probiotics used in combination were *Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium lactis, and Saccharomyces boulardii* (VIAZIS et al. 2022).

Probiotic supplementation therapy has been clinically proven to increase the effectiveness of antibiotics, maintain the host's gastrointestinal microflora, and reduce side effects. However, it is difficult to determine which probiotics are most effective in
treating *H. pylori* based on existing studies, and different combinations of probiotics and antibiotics can produce unparalleled effects. Therefore, we proposed a randomized, double-blind, placebo-controlled clinical trial to evaluate any potential effect of the probiotic *Lactobacillus plantarum* over placebo as additive agents to a standard *H. pylori*-eradication regimen.

**MATERIALS AND METHODS**

This is a randomized, placebo-controlled, double-blind clinical trial with two parallel groups (allocation ratio 1:1) matched by sex and age in dyspeptic patients with gastric infection documented and untreated by *Helicobacter pylori* who were under outpatient follow-up at the Gastroenterology service of the Hospital das Clínicas of the Federal University of Goiás (HC/UFG), a public tertiary referral hospital for the state of Goiás and at the Instituto Goiano de Medicina - Gastroclínica Goiânia LTDA.

Institutional Review Board (IRB) approval was granted by the Research Ethics Committee of the Universidade Federal de Goiás (UFG), Brazil (protocol CAAE 89402417.2.0000.5078). Informed consent was presented to and signed by patients regarding the study's objectives, procedures, risks, and benefits. Recruitment started in June 2017 and it was completed in October 2020.

**Participants and study setting**
The study comprised dyspeptic adults aged between 18 and 70 years, infected with *H. pylori* and confirmed by histopathological examination of gastric biopsies taken in digestive endoscopies, regardless of gender or ethnicity, who were treatment-naïve for *Helicobacter pylori* and who authorized their participation. It was considered *H. pylori* infection was defined in this study by the histopathological presence of *H. pylori* bacilli. Further, endoscopic features of *H. pylori* infection were gastritis induced by *H. pylori* will be classified as predominantly antral or corporeal (PRICE; MISIEWICZ, 1991), and histology was considered the presence of *H. pylori bacilli*, mononuclear cells (lymphocytes, plasma cells, and monocytes) as markers of inflammation, polymorphonuclear leukocytes (neutrophils) as markers of activity, and inflammation and activity were graded using a semi-quantitative score ranging from 0 (none), 1 (minimal), 2 (mild), 3 (moderate) and 4 (severe), by hematoxylin-eosin and Giemsa (PRICE; MISIEWICZ, 1991; STOLTE et al., 1995; PANTOFLICKOVA et al., 2003).
Patients were instructed to answer the questionnaire “Assessment of Gastrointestinal Symptoms” weekly during the first four weeks of the study. A list of possible side effects, such as epigastric pain, diarrhea, altered taste, nausea, and abdominal gas, was handed over to be reported by patients or assistant physician. Moreover, four weeks after therapy, a re-endoscopy was performed by obtaining new gastric biopsies from the antrum and body to evaluate the histopathological alterations and the presence of *H. pylori bacilli*.

Patients with chronic diseases such as diabetes, kidney failure and cirrhosis, malignant neoplasms, gallbladder disorders, peptic ulcer, previous upper digestive tract surgery, previous probiotic therapy in the last month, antibiotics, proton-pump inhibitors, and H2-receptor blocker therapy within one month of starting the study, known allergy to the drugs used, *H. pylori* gastric infection and failure of prior eradication treatment, pregnant women, acquired immunodeficiency syndrome, or who have viral (especially zika, dengue, chikungunya or H1N1) or bacterial infectious diseases were excluded.

The enrollment flowchart of the CONSORT clinical trial and the progress stages can be found in Figure 1.

**Figure 1 - Study timeline**

Interventions

The study groups were distributed as follows (figure 1):

A) Case group – patients who received triple therapy (Amoxicillin 1000mg 2x/day for one week, Clarithromycin 500mg twice/day for one week, Esomeprazole 20mg
2x/day for one week and then once/day for another three weeks) and *Lactobacillus plantarum* strain Lp-G18, orally, in capsules containing 5 x 10^8 CFU, once/day for 4 weeks.

B) Control group - patients who received triple therapy (Amoxicillin 1000mg 2x/day for one week, Clarithromycin 500mg twice/day for one week, Esomeprazole 20mg twice/day for one week and then once/day for another three weeks) and placebo, orally, in capsules, containing maltodextrin, once/day also for 4 weeks.

The probiotic used was *L. plantarum* strain Lp-G18. The capsules contained 5 x 10^8 CFU/mL, and it was imported from China by the importer and distributor LEMMA SUPPLY SOLUTIONS LTDA.

Outcomes

The primary outcome was eradication of *H. pylori*, defined as histopathological absence of *H. pylori bacilli* four weeks after treatment (7 weeks after the end of triple standard therapy).

The secondary outcome was the presence or absence of adverse events, such as diarrhea, taste change/dry mouth, epigastric pain/nausea, and feeling of gas in the abdomen.

Statistical analysis

The software Microsoft Excel version 2020 and Statistical Package for the Social Sciences (SPSS®) 27.0 were used for the input and analysis of the database. Descriptive and bivariate analyses were performed, considering a significance level of 5%. The results were submitted to the following statistical tests, taking into account the nature of the variables studied: measures of central tendency and variability - mean, median, standard deviation (±SD), and mean confidence interval (CI 95 %) evaluated by descriptive statistics; t-Student test was used to comparison between numerical variables that present normal distribution and U-Mann-Whitney test were used to compare variables that do not show normal distribution.

RESULTS

Fifty patients aged between 18 and 74 years (mean 42.7) participated in this clinical study, and 47 concluded this trial (Figure 2). Of these, 27 patients (57.4%) were female (figure 1).
Figure 1 - CONSORT flowchart of clinical trial progress stages.

The characteristics of the participants and gastrointestinal symptoms, according to the allocation in the placebo group or in the group that received the probiotic, are presented in Table 1.

Table 1 Characteristics of the participants and clinical data.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>Placebo (n=22)</th>
<th>Plantarum (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ; n/total (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masculine</td>
<td>20/47</td>
<td>12/22 (54.5)</td>
<td>8/25 (32.0)</td>
<td>0.15*</td>
</tr>
<tr>
<td>Feminine</td>
<td>27/47</td>
<td>10/22 (45.5)</td>
<td>17/25 (68.0)</td>
<td></td>
</tr>
<tr>
<td>Age - years (SD mean)</td>
<td>42.7 (14.8)</td>
<td>46.7 (13.5)</td>
<td>39.1 (15.3)</td>
<td>0.47**</td>
</tr>
<tr>
<td>Epigastralgia - n/total (%)</td>
<td>37/47</td>
<td>17/22 (77.3)</td>
<td>20/25 (80.0)</td>
<td>1.00#</td>
</tr>
<tr>
<td>Completeness n/total (%)</td>
<td>20/47</td>
<td>14/22 (63.6)</td>
<td>13/25 (52.0)</td>
<td>0.56**</td>
</tr>
<tr>
<td>Fullness - n/total duration (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to &lt; 6 months</td>
<td>9/20</td>
<td>5/8 (62.5)</td>
<td>4/12 (33.3)</td>
<td>0.21†</td>
</tr>
<tr>
<td>6 to &lt; 12 months</td>
<td>2/20</td>
<td>0/8 (0.0)</td>
<td>2/12 (16.7)</td>
<td></td>
</tr>
<tr>
<td>≥ 12 months</td>
<td>9/20</td>
<td>3/8 (37.5)</td>
<td>6/12 (50.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher's test ; ** Student's t-test; # chi- square test ; † Fisher- Freemon -Halton test
The total eradication rate was 78.7% (n=37), being statistically similar between the study groups (p = 0.73) (Table 2). Among the 44 patients who reported epigastric pain in the initial consultation, 41 of them (93.2%) showed improvement in the symptom reported in the first four weeks after treatment; there were more reports of progress in the group that received Lactobacillus plantarum without, however, showing a statistically significant difference. Regarding improving the epigastralgia symptom in the first four weeks after treatment, of the 41 patients who reported it, 28 of them (68.3%) remained free of the symptoms after 8 weeks of treatment. The persistence of clinical improvement was also more informed in the group that received probiotics, without presenting a statistically significant difference (Table 2).

Table 2. Clinical and endoscopic post-treatment response of clinical trial participants according to the intervention group (n=47)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Placebo (n=22)</td>
</tr>
<tr>
<td>H. pylori eradication n/total (%)</td>
<td>37/47 (78.7)</td>
<td>18/22 (81.8)</td>
</tr>
<tr>
<td>Improvement of epigastric pain 4 weeks after treatment n/total (%)</td>
<td>41/44 (93.2)</td>
<td>19/21 (90.5)</td>
</tr>
<tr>
<td>Improvement of epigastric pain 8 weeks after treatment n/total (%)</td>
<td>28/44 (63.6)</td>
<td>13/21 (61.9)</td>
</tr>
<tr>
<td>Improvement in Fullness 4 weeks post-treatment n/total (%)</td>
<td>18/19 (94.7)</td>
<td>7/8 (87.5)</td>
</tr>
<tr>
<td>Improvement in Fullness 8 weeks post-treatment n/total (%)</td>
<td>9/19 (47.4)</td>
<td>3/8 (37.5)</td>
</tr>
</tbody>
</table>

Regarding the patients (n=19) who reported gastric fullness in the initial consultation, only one patient in the placebo group did not present improvement of the reported symptom in the first four weeks after treatment (Table 2). Among the 19 patients who reported improvement in the symptom of gastric fullness in the first four weeks after treatment, 9 of them (47.3%) remained free of the symptom after eight weeks of treatment. There was persistent improvement reported in the probiotic group, without statistical evidence (Table 2).
When analyzing the age range of the total number of patients, although there was no statistically significant difference, we observed that eradication in older patients was lower than eradication in younger patients, as shown in Figure 3.

Figure 3 - Box plot of *H. pylori* eradication related to patient age (n=47).

A detailed analysis of patients aged up to 45 years showed that the eradication rate in patients who received the probiotic was 93.8% against only 70% in patients who received the placebo (p = 0.046), as seen in Table 3.
Table 3. Comparison of *H. pylori* eradication in all patients and patients under 45 years of age.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Placebo</th>
<th>Plantarum</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All you patients (n=47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eradicated</td>
<td>18/22 (81.8)</td>
<td>19/25 (76.0)</td>
<td>0.730</td>
</tr>
<tr>
<td>No eradicated</td>
<td>4/22 (18.2)</td>
<td>6/25 (24.0)</td>
<td></td>
</tr>
<tr>
<td>Patients &lt; 45 years old (n=26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eradicated</td>
<td>7/10 (70.0)</td>
<td>15/16 (93.8)</td>
<td>0.046</td>
</tr>
<tr>
<td>No eradicated</td>
<td>3/10 (30.0)</td>
<td>1/16 (6.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher’s exact test

Regarding the main adverse events related to the treatment, it was observed that, of the 25 patients allocated to the Probiotic group, only 3 (12.0%) had abdominal pain, and none reported diarrhea or nausea. In relation to the placebo group, 10 of the 22 patients (45.5%) developed abdominal pain. 7 (31.8%) reported diarrhea, and 3 (13.6%) developed nausea after starting treatment (figure 4).

Figure 4. Distribution of treatment-related adverse events in the Probiotic and Placebo groups.

**DISCUSSION**

This randomized, double-blind, placebo-controlled clinical trial of the use of the probiotic *Lactobacillus plantarum* as adjuvant therapy to antibiotic therapy in the treatment of *Helicobacter pylori* demonstrated a higher success rate in eradicating *H.*
pylori in the age group below 45 years old when compared I'm the older age group and less adverse effects, indicating the therapeutic potential of the association of Lactobacillus plantarum with triple antibiotic therapy in patients younger than 45 years old.

The present study showed a total eradication rate of Helicobacter pylori of 78.7%. 81.8% in the placebo group and 76% in the group that received the probiotic Lactobacillus plantarum, without, however, having statistically significant difference between the two groups. Our result agrees with the literature, where the eradication rate is between 60 and 80% (HOMAN; OREL, 2015). Similar to our findings, Noorbakhsh et al., in a 2022 study that evaluated the efficacy and safety of quadruple therapy with furazolidone-bismuth for the eradication of Helicobacter pylori with or without probiotic supplementation achieved a total eradication rate of 80.5%, with an eradication rate of 84% in the probiotic group and 77% in the placebo group (p=0.2).

In the present study, we also observed that eradication in older patients was lower than eradication in younger patients, considering the total number of patients.

When analyzing the total number of patients aged up to 45 years, we observed that the eradication rate was 84.6% against 78.72% in the total number of patients. Moreover, in patients aged up to 45 years who received the probiotic, the eradication rate was 93.8% against only 70.0% in patients of the same age group who received the placebo (p = 0.046). Our hypothesis about such data is that in young adults, the use of adjuvant therapy with Lactobacillus plantarum may be more effective than in older individuals. In this context, GOMEZ-RAMIREZ et al., in a 2021 systematic review, postulate the hypothesis that age may be a risk factor for worsening gastric dysbiosis, further aggravated by Helicobacter pylori infection. Additionally, it is accepted that H. pylori infection is acquired mainly in childhood, particularly in the first five years of life, and, unless treated, it can remain for decades and probably for the entire life of the individual (KODAIRA et al., 2002). From our point of view and as explained in our results, in young adults, the duration of the infection would present the potential for less alteration in the biofilm and, consequently, greater ease of eradication.

H. pylori infection usually begins with chronic mononuclear inflammation that progresses to varying degrees of acute neutrophilic inflammation. Due to the progression of inflammation, gastritis progresses to atrophic gastritis over the years, which is characterized by the loss of normal mucous glands in the body and antrum. Thus, chronic inflammation is closely associated with neutrophilic inflammation and cytotoxicity of infecting H. pylori strains (SIPPONEN; MAAROOS, 2015). Therefore, we can infer that
the sooner the treatment is instituted, the greater the chance of avoiding the progression described by the authors above, which strengthens our findings. Furthermore, a study carried out with pediatric patients showed that the intestinal microbiota is modified by *Helicobacter pylori* infection, noting an abundance of the Bacteroidaceae, Enterobacteriaceae and Porphyromonadaceae families, and Bacteroides, Parabacteroides, Streptococcus, and Lactococcus genera when compared to the microbiota of healthy individuals. While the families Bifidobacteriaceae, Lactobacillaceae, and Lachnospiraceae showed lower relative abundance in patients positive for *Helicobacter pylori* compared to healthy individuals (YANG, L. et al. 2019). Such data reinforce data that the use of adjuvant therapy with Lactobacillus Plantarum associated with triple antibiotic therapy would contemplate the recomposition of the Lactobacillaceae family, contributing to the improvement of dysbiosis and consequently to the eradication of *Helicobacter pylori*.

Among the limitations presented by the present study, the fact that it was not a multicenter study stands out. However, its rigorous methodology will undoubtedly allow future systematic reviews to contribute to a clearer understanding of the role of probiotics in eradicating *Helicobacter pylori*.

**CONCLUSION**

The results indicate the potential of using probiotics as an adjuvant in eliminating *Helicobacter pylori* and instigate investigations of the greater potential for synergism of probiotics in younger age groups.

**FUNDING**

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**REFERENCES**


