Antimicrobial Effects of Aqueous and Hydro-Alcoholic *Plantago psyllium* leaf extracts on the Experimental Infection of *Helicobacter pylori* in a Rat Model

Shima Keshavarzi¹,², Masood Sepehrimanesh¹*, Bahman Mirzaei¹, Omid Koohi-Hosseinabadi¹, Zeynab Sardari¹, Mohammad Mehdi Sajjadpur²

1. Dept. of Microbiology, Fars Sciences and Research Branch, Islamic Azad University, Shiraz, Iran
2. Dept. of Microbiology, Shiraz Branch, Islamic Azad University, Shiraz, Iran
3. Dept. of Biology, University of Louisiana at Lafayette, Louisiana, USA
4. Dept. of Medical Microbiology and Virology, Faculty of Medicine, Mazandaran University of Medical Science, Sari, Iran
5. Dept. of Medical Microbiology and Virology, School of Medicine, Zanjan University of Medical Science, Zanjan, Iran
6. Central Laboratory, Shiraz University of Medical Sciences, Shiraz, Iran
7. Gastroenterology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

**ABSTRACT**

**Background & Objective:** *Plantago psyllium* has long been utilised as a medicinal agent worldwide. The current study aims to evaluate the *in vivo* antimicrobial effects of aqueous *P. psyllium* and a hydro-alcoholic extract of *P. psyllium* on *Helicobacter pylori* as well as the effects of these extracts on liver and kidney in a rat model.

**Materials & Methods:** A total number of 50 female Sprague Dawley rats were experimentally infected (Except for the negative control group). The rats were divided into five groups of 10. The groups were treated by Aqueous and Hydro-Alcoholic *P. psyllium* leaf extracts. Subsequently, *H. pylori* antigens which were present in the in rats’ stool were measured using a serological assay. To diagnose the pathogenicity of the kidney and liver, blood samples which had been taken before and after treatment, were tested for renal and hepatic enzymes by Analyzer Electrolyte and Sysmex KX-21N.

**Results:** Following the amoxicillin treatment, the levels of aqueous and hydro-alcoholic extracts of the treated rats were compared with those of the control group, and a significant difference was observed (*P*<0.05). Antigen decrease was observed in all of the groups treated with amoxicillin (*P*=0.0004), aqueous, and hydro-alcoholic extracts (*P*<0.05). Antigen decrease in the stool was observed in all groups, which indicates that treatment with the herbal extract could affect the infected rats.

**Conclusion:** *P. psyllium* hydro-alcoholic extract can be applied as a selective treatment for *H. pylori* infection. Hopefully, experiments suggested that the mentioned extracts could positively affect the process of disease recovery.

**Keywords:** Aqueous and Hydro-Alcoholic extracts, *Helicobacter pylori*, *Plantago psyllium*

**Introduction**

*Helicobacter pylori* colonizes half of the world’s population and contributes to gastritis and gastrointestinal ulcer disease increasing the risk of malignant gastrointestinal cancer (1). The onset of gastric adenocarcinoma occurs with the progression of chronic superficial gastritis to chronic gastric atrophy. Furthermore, *H. pylori* is a risk factor for gastric cancer (2). It is estimated that 14,500 patients (>65 age) pass away due to *H. pylori* infections annually (2). Antibiotics such as clarithromycin, metronidazole, amoxicillin, and tetracycline are used to treat *H. pylori* infections. *H. pylori* resistance rate has been estimated to range from 15% to 78%. According to previous reports, the antibiotic resistance of the mentioned bacterium against amoxicillin and other antibiotics has increased [4]. Multi-drug resistant (MDR) *H. pylori* isolates were screened >75% in Iran (3). *Plantago psyllium* is widely used as a traditional herbal medicine and a functional food in Asian counties (4). Polysaccharide, the main biological active compartment in *Plantago* spp., has a variety of biological effects including gastrointestinal functions and antioxidant, immunomodulatory, antitumor, and hypoglycemic activities. Variety
of biological and pharmacological functions of psyllium macromolecules have been confirmed (4). *P. psyllium* can be found in almost every region of the world and people have used *P. psyllium* to treat gastric and peptic ulcers for a very long time. This herb has had medicinal uses in Iranian traditional medicine as well (5-7). *P. psyllium* seeds contain 10% mucilage, which produces D-xyllose, arabinose, D-glucose, and D-galacturonic acid through hydrolysis (8). In the United States, a medicine called “Metamucil” is produced from the laxative, but researchers suggest that *P. psyllium* is also effective in reducing cholesterol and glucose levels (9). Anti-bacterial properties of herbs against *H. pylori* infection were indicated in some published studies (10). Although there are some reports about *P. psyllium* anti-bacterial properties (11), no study has investigated *H. pylori* infections. Therefore, investigation of the antimicrobial effects of aqueous and hydroalcoholic extracts of *P. psyllium* on the infected experimental rat groups, is the main purpose of the current study.

**Materials and Methods**

**Preparation of Aqueous and Hydro-alcoholic Extracts of *P. psyllium***

Extraction was carried out in the faculty of pharmacy under the supervision of experts as follows: fresh *P. psyllium* was provided from Shiraz, a city in south-central Iran. The species were matched with the German Digital Herbarium in the Faculty of Pharmacy at Shiraz University of Medical Sciences. It was found that the species had a herbarium number as B-W0298230 (9). To prepare the aqueous and hydro-alcoholic extracts of *P. psyllium*, the plants were dried at room temperature for five days and were then powdered. Using the percolation method, 100 g of powdered *P. psyllium* leaf was poured into the percolator and soaked in distilled water for 24 h. Another 100 g of powdered *P. psyllium* was soaked in another percolator device with one litre of 70% ethanol for 72 h. After filtering and evaporation, the obtained gelatinous extracts were reduced under pressure in the rotary device and were finally prepared as powder (12, 13).

**Preparation and Culture of Bacteria**

Gastric biopsy samples were taken by endoscopy from patients who referred to Namazi hospital, Shiraz. For early isolation of bacteria, agar enriched with yeast extract of Columbia culture medium (Merck, Germany) was enriched with 10% defibrinated horse blood, 10 mg/L vancomycin, 10 mg/L amphotericin B, 5 mg/L cesfudolin, and 5 mg/L trimethoprim and incubated at 37°C (14). According to previously published research, Urease test was performed on biopsy samples, which were examined for the presence or absence of *H. pylori* (15).

**Confirmation of *H. pylori* Isolates**

According to the previously published research, the isolated strains were precisely reconfirmed taking advantage of traditional biochemical tests including gram staining, urease activity, oxidase, and catalase enzymes production (14).

**Taking Care of Animals**

Fifty female rats of Sorague Dawly with a weight range of 200±20 g were randomly divided into five groups. Rats were kept in standard conditions for relative temperature of 22±2% and received water and food every day. Consumption doses were calculated based on previous studies and advice of traditional medicine experts (4).

**Infection and Treatment**

All groups, except for the negative control, received (orally) 0.5 ml of a suspension containing 2×10⁷ colony-forming unit per millilitre (CFU/ml) of *H. pylori* on average every day in the morning for 7 days (4, 16). After 48 weeks of infections, groups were treated for 26 days. *P. psyllium* extracts were performed as single dosage in 300mg final concentration for experimented groups. Some study findings related to the herbal extract in diarrhea showed that higher doses (500 and 1,000 mg/kg), had antisecretory and antidiarrheal activity (17).

The experimental groups were as follows:

**G-1**: Negative control group, which was not subject to infection and treatment at all.

**G-2**: Amoxicillin antibiotic group, in which infection was treated with 500 mg of amoxicillin.

**G-3**: Aquinas extract group, in which, after infecting, the rats were treated with aqueous extract of *P. psyllium* at a concentration of 300 mg/kg of body weight.

**G-4**: Hydro-alcoholic extract group, in which, after infecting, the rats were treated with hydro-alcoholic extract of *P. psyllium* at a concentration of 300 mg/kg of body weight. No cosolvents or surfactant were added to the extract.

**G-5**: Positive control group, in which rats were not treated after infecting.

**Biochemical Analysis of Rats’ Blood in Each Group**

Sampling (blood and stool) from the treated groups was immediately performed eight weeks after infections. Sera were tested in terms of renal and hepatic functions by assessing aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), creatinine, and blood urea nitrogen (BUN) according to the previously published research (18) using an Analyzer Electrolyte, Hitachi 911 (Roche Company, Switzerland) with Pars-Azmoon Commercial Kits (Pars-Azmoon Laboratories LTD, Tehran, Iran) and Sysmex KX-21N (Sysmex Corporation, Japan) according to the manufacture’s manual (Fig. 2, 3). Bouin solution, ethanol, and chloroform were obtained from Sigma-Aldrich Company, USA.

**Enzyme-linked Immunosorbent Assay (ELISA)**

ELISA was done taking advantage of polyclonal antibody-based Premier Platinum HpSA test, later HpSA (Meridian Inc., Cincinnati, USA) was performed based on the manufacturer’s instructions.
Qualitative ELISA was to be performed immediately before and after the treatment, stool sampling was performed for the experimental groups to detect *H. pylori* antigens. Briefly, following the kit manufacturer’s recommended procedure, the plates were washed with washing buffer [0.05 % (v/v) Tween 20 in phosphate buffer saline (PBS)] twice. The plates were incubated for 2 h at 37°C. Blocking was carried out using a PBS/Tween 20 solution containing 5% bovine serum albumin (BSA) as a blocking buffer. After filling blocking and washing, 50 µl of homogenate rat stool samples were added to the wells in duplicate. The plates were incubated for 1 h at 37°C, washed three times, and incubated with HRP-conjugated anti-mouse immunoglobulin (IgG) (Sigma, USA), which was diluted to 1:10,000 (as a secondary antibody) at 37°C for 2 h. The plates were then washed as described above, and the enzymatic activity was measured subsequent to adding 100 µl of tetramethylbenzidine (TMB) substrate. After 30 min of subjoining of 100 µl 2 N H₂SO₄, the reaction was stopped. Finally, the optical density (OD) of each well was determined as 405 nanometres, taking advantage of an ELISA microplate reader (Anthos 2020, United Kingdom). Detailed data are given in Figure 1A.

**Ethical Code**

All animal experiments were done in compliance with the enlarged ethical statement and approved by the ethics committee of Shiraz University of Medical Sciences. All tests were performed complying the enlarged ethical (Human and Animal) statement (95-01-13-11069). The ethics committee approved both the human and the animal experimentation.

**Results**

Extraction was done based on previously published work using the species that had a herbarium number of B-W0298230. A dried extract was then used to prepare the aqueous and hydro-alcoholic extracts (9). The experimental rat groups were infected, following a previously published procedure (4, 16).

Antibody-mediated ELISA was used to determine the *H. pylori* antigen in the homogenate rats’ stool. Following the amoxicillin treatment, the levels of aqueous and hydro-alcoholic extracts of the treated rats were compared with those of the control group, and a significant difference was observed (*P*<0.05). A decrease in antigens was observed for all tested groups treated with amoxicillin (*P*=0.0004), aqueous, and hydro-alcoholic extracts (*P*<0.05) (Fig. 1A). After two weeks of treatment with medicine extracts, all groups showed a describing amount of *H. pylori* antigen in their stool, thus indicated the efficacy of the medicines.

Additionally, in the liver function test (LFT) assay, a significant difference (especially in aquatic extract) was observed in the levels of liver (AST and ALP) and renal damage markers, including BUN and creatinine (*P*<0.05). Significant differences were also seen in the levels of liver enzymes including ALT (*P*=0.00001), AST (*P*=0.00001), ALP (*P*=0.0005), and total protein (*P*<0.00001) content of the serum before and after treatment. This amount was also significant when different treated groups were compared with each other after treatment with medicine extracts. By comparing the amounts of liver and renal indicators among the treated groups, it was found that the amounts of ALP (as an example) to amoxicillin/aqueous, amoxicillin/hydro-alcoholic, and hydro-alcoholic/aqueous were significant (*P*<0.05) after treatment. A statistical analysis showed that blood samples especially to aquatic extract were significantly different in terms of factors that affect the liver and kidney. Furthermore, all of the findings were statistically significant when compared with positive control after treatment (Figs. 1, 2, and 3).

**Figure 1.** Difference in fecal homogeneity antigen A) and Total protein in tested groups B). The error bar is representative of the mean±sd (n=3).
Figure 2. Biochemical analysis of liver enzymes (ALP, and AST) to tested sera in treated rat groups. A) The amount of ALP in experimental groups B) The differences of AST in studied groups. The error bar is representative of the mean±sd (n=3).

Figure 3. Analysis of biochemical factors in treated and untreated rat sera, A) the creatinine level in various groups, and B) the BUN differences in studied. The error bar is representative of the mean±sd (n=3).

Discussion

Antibiotics are often used to treat *Helicobacter* infections, and common antibiotics cause severe damage to the normal flora of the digestive system, which, in the long term, causes malnutrition and complicated diseases (19). *P. psyllium* is a valuable medicinal plant that researchers have always been interested in knowing more about. Numerous studies have been performed on the effects of this plant and its therapeutic role in constipation, diarrhea, ulcerative colitis, irritable bowel syndrome, colon cancer, diabetes, and hypercholesterolemia (20-22).

Considering the long history of the effects of *P. psyllium* on treating microbial diseases in humans and animals, in the current research, the therapeutic features of aqueous and hydro-alcoholic extracts of *P. psyllium* were investigated in rat models. The findings showed that both extracts had significant therapeutic effects, and additional experiments demonstrated that these extracts have minor adverse effects on renal and hepatic functions. Given the emergence of MDR *H. pylori* isolates and the adverse effect of the mentioned drug of choice on liver and kidney functions, it seems appropriate to consider the use of herbal and natural ingredients to treat *H. pylori*-associated infections (3).

The protective effect of pectic polysaccharide in *P. psyllium* against systemic *Streptococcus* infections has been shown in experimental rats. This protective effect appeared to be due to the stimulation of the immune system. Other antibacterial and anti-Candida effects of pectic polysaccharide in *P. psyllium* were confirmed in another review (23). In a study investigating the antimicrobial activity of nanoparticles produced in the
examined *P. psyllium*, findings indicated that the highest and the lowest concentrations of inhibitors in *Staphylococcus aureus* were 100 mg/ml and 12.5 mg/ml, respectively. These results showed that *P. psyllium* has good antimicrobial functioning in the treatment of many common antibiotic-resistant infections (24). In a study conducted by Taheri et al., on the effects of hydro-alcoholic extracts of *P. psyllium* on *S. aureus*, the researchers found that these extracts had a significant effect on *S. aureus* bacteria in vitro (25). In the present study, the levels of fecal antigens and antibodies against *H. pylori* were reduced in all samples. This was also observed in *Salmonella typhimurium* bacteria (11). At last, although the mentioned herbal extract is suitable for *H. pylori* treatment, according to the obtained results especially in aquatic extract, this cocktail has an adverse effect on liver and renal functions compared to the hydroalcoholic extract.

**Conclusion**

Antimicrobial resistant patterns of human pathogens are essential problems, resulting in the need for novel antimicrobial resources such as plants. This study confirmed that Aqueous and Hydro-Alcoholic *P. psyllium* leaf extracts could be considered as alternative choice treatments due to the lack of renal and hepatic side effects and their proper antimicrobial effects in experimental *H. pylori* infection in a rat model. However, further research on more samples and human models are recommended.

**Limitations**

In the current study, herbal antibacterial effects were not measured in bacterial culture media such as agar well diffusion. Moreover, *H. pylori* was not measured in the stool samples before and after treatment because of financial restrictions. Therefore, more studies are needed to make a precise evaluation of the bactericidal effects of the herbal extract on *H. pylori*.

**Abbreviations**

*H. pylori*: *Helicobacter pylori*  
*P. psyllium*: *Plantago Psyllium*  
MDR: multi-drug resistant  
CFU/ml: colony-forming unit per millilitre  
AST: aspartate aminotransferase  
ALT: alanine aminotransferase  
ALP: alkaline phosphatase  
TP: total protein  
BUN: blood urea nitrogen  
ELISA: Enzyme-linked immunosorbert assay  
OD: optical density  
BSA: bovine serum albumin  
IgG: immunoglobulin G  
TMB: tetramethylbenzidine  
PBS: phosphate buffer saline  
ANOVA: analysis of variance  
LFT: liver function test

**Declarations**

Ethics approval and consent to participate: In this study, all ethics including Ethics and Consent to participate have been collected in the research. Informed consent, (written) was obtained from all participants for the ‘Gastric biopsy samples’. Inbred Sprague Dawley rats were purchased from the Research Institution of Pasteur, Karaj, Iran. They were housed in standard cages (at 23-25°C and 60–70% humidity under a 12 h light/dark cycle) with free access to standard diet and water, one week before the experiment. Euthanasia was performed using 2-5% halogenated ether which is an inhalant anesthetic, according to Evaluation of the Aesthetics of Physical Methods of Euthanasia of Anesthetized Rats, 2011 protocol.

In this survey, Ethics and Consent to participate from their parents have been collected. All analyses were performed using multiple-group analysis of variance (ANOVA) by Graph Pad software (Chicago, IL, USA). P-value≤0.05 was regarded as statistically significant.

**Consent for publication**

Not applicable

**Competing Interests**

The authors declare no competing interests.

**Authors’ contributions**

All authors have read and approved the manuscript. Contributions of the authors in this study were as follow:

S K: Performing laboratory tests  
M S M: Designing the study, and writing the manuscript  
B M: Interpretation of results and rewriting the manuscript  
O K H A: Sampling  
Z S: Performing laboratory tests  
M M S: Performing laboratory tests
Funding

Not applicable.

Acknowledgments

The authors wish to express gratitude to their colleagues in Shiraz University of Medical Sciences, Zanjan University of medical sciences, and Namazi hospital in Shiraz province, Iran.

Conflict of Interest

The authors announce that they have no conflict of interest.

References

1. Magalhães Queiroz DM, Luzzia F. Epidemiology of Helicobacter pylori infection. Helicobacter. 2006;11:1-5. [DOI:10.1111/j.1478-405X.2006.00429.x]

2. Lawrence RS, Durch JS, Stratton KR. Vaccines for the 21st century: a tool for decisionmaking: National Academies Press; 2001.

3. Farzi N, Yadegar A, Sadeghi A, et al. High prevalence of antibiotic resistance in Iranian Helicobacter pylori isolates: importance of functional and mutational analysis of resistance genes and virulence genotyping. J Clin Med. 2019;8(11):2004. [DOI:10.3390/jcm8112004]

4. Zhang XL, Jiang AM, Ma ZY, et al. The synthetic antimicrobial peptide pexiganan and its nanoparticles (PNPs) exhibit the anti-Helicobacter pylori activity in vitro and in vivo. Molecules. 2015;20(3):3972-85. [DOI:10.3390/molecules20033972]

5. Ahmadi R, Kalbasi-Ashhari A, Oromiehie A, Yarmand M-S, Jahandideh F. Development and characterization of a novel biodegradable edible film obtained from psyllium seed (Plantago ovata Forsk). J Food Engineer. 2012;109(4):745-51. [DOI:10.1016/j.jfoodeng.2011.11.010]

6. Hajjhashemi V, Ghanmadi A, Hajiloo M. Analgesic and anti-inflammatory effects of Rosa damascena hydroalcoholic extract and its essential oil in animal models. Iran J Pharmaceutical Res. 2010;9(2):163.

7. Hajjhashemi V, Sajjadi SE, Heshmati M. Analgesic and anti-inflammatory properties of Heracleum persicum essential oil and hydroalcoholic extract in animal models. J Ethnopharmacol. 2009;124(3):475-80. [DOI:10.1016/j.jep.2009.05.012]

8. Ladjevardi ZS, Gharihzedehi SMT, Mousavi M. Development of a stable low-fat yogurt gel using functionality of psyllium (Plantago ovata Forsk) husk gum. Carbohydrate Polymers. 2015;125:272-80. [DOI:10.1016/j.carbpol.2015.02.051]

9. Abou Azar S, Wehbe MR, Jamali S, Hallal A. Small bowel obstruction secondary to a Metamucil bezoar: Case report and review of the literature. Case Rep Surg. 2017;2017: 2702896. [DOI:10.1155/2017/2702896]

10. Yousef-Nezhad H, Hejazi N. Anti-bacterial properties of herbs against Helicobacter Pylori infection: A review. Int J Nutr Sci. 2017;2(3):126-33.

11. Sepehri Z, Nasiri AA, Azizi A, Golshani F, Sargazi A. Antibacterial effects of ethanol extract of Plantago psyllium on antibiotic-resistant Salmonella typhimurium. J Zabol Univ Med Sci Health Serv. 2016;7(4):1-8.

12. Graham DY, Malaty HM, Evans DG, Evans Jr DJ, Klein PD, Adam E. Epidemiology of Helicobacter pylori in an asymptomatic population in the United States: effect of age, race, and socioeconomic status. Gastroenterol. 1991;100(6):1495-501. [DOI:10.1016/0016-5085(91)90644-Z]

13. Lieber CS. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. Mt Sinai J Med. 2000;67(1):84-94

14. McColl KE. Helicobacter pylori infection. New Eng J Med. 2010;362(17):1597-604. [DOI:10.1056/NEJMcp1001110]

15. Pastukh N, Binyamin D, On A, Paritsky M, Peretz A. GenoType® Helico DR test in comparison with histology and culture for Helicobacter pylori detection and identification of resistance mutations to clarithromycin and fluoroquinolones. Helicobacter. 2017;22(6):e12447. [DOI:10.1111/hel.12447]

16. Trivedi MK, Gangwar M, Mondal SC, Jana S. Immunomodulatory properties and biomarkers characterization of novel Withania somnifera based formulation supplemented with minerals in Sprague Dawley rats. Orient Pharm Experiment Med. 2017;17(1):59-69. [DOI:10.1007/s13596-016-0255-5]

17. Mehmood MH, Aziz N, Ghayur MN, Gilani AH. Pharmacological basis for the medicinal use of psyllium husk (Ispaghula) in constipation and diarrhea. Digest Dis Sci. 2011;56(5):1460-71. [DOI:10.1007/s10620-010-1466-0]

18. Radfar MH, Zarandi MB, Bamorovat M, Kheirandish R, Sharifi I. Hematological, biochemical and pathological findings in goats naturally infection with Cysticercus tenuicollis. J
19. Graham DY, Fischbach L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. Gut. 2010;59(8):1143-53. [DOI:10.1136/gut.2009.192757]

20. Mobin M, Rizvi M. Polysaccharide from Plantago as a green corrosion inhibitor for carbon steel in 1 M HCl solution. Carbohydrate Polymer. 2017;160:172-83. [DOI:10.1016/j.carbpol.2016.12.056]

21. Pouladi S, Bagherpour Borazjani A, Motamed N, et al. A Survey on the effect of psyllium on serum levels of triglycerid and lipoproteins. Iran South Med J. 2009;11(2):139-46.

22. Singh B. Psyllium as therapeutic and drug delivery agent. Int J Pharmaceut. 2007;334(1-2):1-14. [DOI:10.1016/j.ijpharm.2007.01.028]

23. Hetland G, Samuelsen A, Loslash V, et al. Protective effect of Plantago major L. Pectin polysaccharide against systemic Streptococcus pneumoniae infection in mice. Scand J Immunol. 2000;52(4):348-55. [DOI:10.1046/j.1365-3083.2000.00793.x]

24. Bokaeian M, Fakheri BA, Mohasseli T, Saeidi S. Antibacterial activity of silver nanoparticles produced by plantago ovata seed extract against antibiotic resistant Staphylococcus aureus. Int J Infect. 2015;2(1):e22854. [DOI:10.17795/iji-22854]

25. Taheri A, Seyfan A, Jalalinezhad S, Nasery F. Antibacterial effect of Myrtus communis hydro-alcoholic extract on pathogenic bacteria. Zahedan J Res Med Sci. 2013;15(6):19-24.