Peer Review File

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Reviewer A

This manuscript contains a description of the experiment carried out to determine the effect of the fucoidan plant drink in eradicating *Helicobacter pylori* (Hp) in humans. A total of 122 patients with confirmed Hp infection were enrolled. The composition of the fucoidan plant drink and experimental conditions are well described that opens a possibility for future testing of the results by other researchers. In my opinion, this study is interesting because positive therapeutic effect was reached without use of antibiotics. The general level of this study is high and manuscript could be considered for publication after minor revision reasonable to increase the paper quality. My several corrections proposed for the text are listed below for author consideration.

Page 2

Recently the anti-adhesive, antioxidant, antitoxin, immunomodulatory, anti-coagulant, and anti-infective activities of fucoidan, a polysaccharide extracted from brown seaweeds, have been widely studied and showed promise.

Recently, the anti-adhesive, antioxidant, antitoxin, immunomodulatory, anti-coagulant and anti-infective activities of fucoidan, a polysaccharide extracted from brown seaweeds, were widely studied, and the results showed promise.
Reply: Revised as suggested.

Page 2

Our present clinical study was designed to evaluate the effectiveness of Lewuyou®, a fucoidan plant drink (FPD) in eradicating Hp in humans.

Our present clinical study was designed to evaluate the efficiency of Lewuyou®, a fucoidan plant drink (FPD), in eradicating Hp in humans.
Reply: Revised as suggested.

Page 2

The Hp eradication rate and clearance rate were 77.6% (66/85) and 20.0% (17/85) respectively, after 4 weeks of FPD consumption and 80.5% (33/41) and 26.8 (11/41) , respectively, after 8 weeks of consumption.

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Reply: Revised as suggested.

Page 2

The 4- and 8-week protocols of FPD consumption was safe and effective at reducing Hp load on the gastric mucosa, with Hp eradicated in the majority of participants.

The 4- and 8-week protocols of FPD consumption were safe and effective at reducing Hp load on the gastric mucosa, with Hp eradicated in the majority of participants.
Reply: Revised as suggested.

Page 3

The infection rate among children is also Considerably high (2).

The infection rate among children is also considerably high (2).
Reply: Revised as suggested.

Page 4

The development of effective, less toxic eradication therapies remains a key research topic (14).

In this relation, for generality, one recent paper could be cited additionally:
Int. J. Mol. Sci. 24 (2023) 11906
Reply: Revised as suggested.

Page 4

Recently, the anti-adhesive, antioxidant, antitoxin, immunomodulatory, anti-coagulant, and anti-infective activities of *Ascophyllum nodosum* (knotted wrack; a type of brown seaweed that contains fucoidan) extracts have been extensively studied. It has been proposed that fucoidan

may be classified as a new drug that could be included in therapeutic regimens for Hp eradication (15).

Recently, the anti-adhesive, antioxidant, antitoxin, immunomodulatory, anti-coagulant, and anti-infective activities of *Ascophyllum nodosum* (knotted wrack; a type of brown seaweed that contains fucoidan) extracts were extensively studied. It was proposed that fucoidan may be classified as a new drug that could be included in therapeutic regimens for Hp eradication (15).
Reply: Revised as suggested.

Page 4

We aimed to assess whether a drink containing fucoidan was clinically valuable for eradicating Hp and lowering Hp load.

This our study was aimed at the assessment whether a drink containing fucoidan was clinically valuable for eradicating Hp and lowering Hp load.
Reply: Revised as suggested.

Page 4

It's categorized as a "food product" and containing the following main ingredients: water, radish seed compounded plant beverage (including water, isomalto-oligosaccharide, glucose, yam, radish seed, and hawthorn), apple juice concentrate, broccoli powder (including maltodextrin and broccoli), fucoidan (200 mg), *Lycium ruthenicum Murray* powder, instant green tea powder, erythritol, pectin, citric acid, DL-malic acid, sucralose, stevioside, and edible flavors and fragrances.

It is categorized as a "food product" and containing the following main ingredients: water, radish seed compounded plant beverage (including water, isomalto-oligosaccharide, glucose, yam, radish seed, and hawthorn), apple juice concentrate, broccoli powder (including maltodextrin and broccoli), fucoidan (200 mg), *Lycium ruthenicum Murray* powder, instant green tea powder, erythritol, pectin, citric acid, DL-malic acid, sucralose, stevioside, and edible flavors and fragrances.
Reply: Revised as suggested.

Page 6

The participants' activities (including diets) were not restricted during the study period.

The participant activities (including diets) were not restricted during the study period.
Reply: Revised as suggested.

Page 7

AEs that affected the patient's quality of life including abdominal pain, diarrhea, constipation, dizziness, dysgeusia, headache, anorexia, nausea, vomiting, and rash were also recorded.

AEs that affected the patient quality of life, including abdominal pain, diarrhea, constipation, dizziness, dysgeusia, headache, anorexia, nausea, vomiting and rash, were also recorded.
Reply: Revised as suggested.

Page 8

Symptoms including abdominal distension, nausea, belching, and acid reflux were improved in some patients.

Symptoms, including abdominal distension, nausea, belching and acid reflux, were improved in some patients.
Reply: Revised as suggested.

Page 8

In a post-bone marrow transplant, a patient with intestinal rejection combined with cytomegalovirus (CMV) infection who had been producing 30 bloody, watery stools per day, the frequency of defecation decreased to 4 times/day after FPD consumption.

In a post-bone marrow transplant, for a patient with intestinal rejection combined with cytomegalovirus (CMV) infection, who had been producing 30 bloody, watery stools per day, the frequency of defecation decreased to 4 times/day after FPD consumption.
Reply: Revised as suggested.

Page 9

For example, epinecidin-1, a multifunctional antimicrobial peptide produced by *Epinephelus coioides*, has been shown to have the potential to replace antibiotics with its significant efficacy in fighting against *S. aureus* and Hp (18).

For example, epinecidin-1, a multifunctional antimicrobial peptide produced by *Epinephelus coioides*, was shown to have the potential to replace antibiotics with its significant efficacy in fighting against *S. aureus* and Hp (18).
Reply: Revised as suggested.

Page 10

Fucoidan is that fucoidan could interfere with the linkage/attachment of Hp to gastric mucosal cells.

This sentence is unclear.
Reply: Revised, “that fucoidan could” → “believed to” .

Page 10

Furthermore, Cai et al.’s research demonstrated that a combination of fucoidan polysaccharides and evening primrose extract (FEMY-R7), exhibited complete inhibition of Hp *in vitro* at a concentration of 100 μg/mL.

Furthermore, it was demonstrated in Cai et al. research that a combination of fucoidan polysaccharides and evening primrose extract (FEMY-R7) exhibited complete inhibition of Hp *in vitro* at a concentration of 100 μg/mL.
Reply: Revised as suggested.

Page 10

Animal experiments revealed that FEMY-R7 cleared gastric mucosal infection by directly killing the bacteria and preventing their adhesion and invasion (26).

Animal experiments revealed that FEMY-R7 cleared gastric mucosal infection by direct killing the bacteria and preventing their adhesion and invasion (26).
Reply: Revised as suggested.

Page 10

In a clinical study, humans confirmed to be infected with Hp, were orally administered twice daily with a capsule containing 150 mg FEMY-R7 for 8 weeks.

In a clinical study, humans confirmed to be infected with Hp were orally administered twice daily with a capsule containing 150 mg FEMY-R7 for 8 weeks.
Reply: Revised as suggested.

Page 10

Additionally, observations on healthy volunteers indicate that fucoidan can be absorbed and metabolized by the human body, with absorption being correlated to the frequency of Hp infection and seaweed product usage (28,29).

Additionally, the observations on healthy volunteers indicate that fucoidan can be absorbed and metabolized by the human body, with absorption being correlated to the frequency of Hp infection and seaweed product usage (28,29).
Reply: Revised as suggested.

Page 11

A study on mice (Tomori et al.) revealed different doses of fucoidan were administered orally continuously for 6 weeks, immune cell proliferation, interleukin (IL)-2, macrophage phagocytes, and serum antibodies (IgM, -G, -A) increased significantly.

A study on mice (Tomori et al.) revealed that when different doses of fucoidan were administered orally continuously for 6 weeks, immune cell proliferation, interleukin (IL)-2, macrophage phagocytes, and serum antibodies (IgM, -G, -A) increased significantly.
Reply: Revised as suggested.

Page 11

Fucoidan dose-dependently inhibited the proliferation of KSHV-infected PEL cell lines, highlighting the anti-PEL actions of fucoidan, the mechanism is believed to be associated with its immunomodulatory effects (33).

Fucoidan dose-dependently inhibited the proliferation of KSHV-infected PEL cell lines, highlighting the anti-PEL actions of fucoidan, and the mechanism is believed to be associated with its immunomodulatory effects (33).
Reply: Revised as suggested.

Page 11

Some Chinese studies have investigated the clinical effectiveness of both STT and quadruple therapy.

Some studies implemented in China have investigated the clinical effectiveness of both STT and quadruple therapy.
Reply: Revised as suggested.

Page 11

In contrast, the clearance rate of Hp was slightly lower after 8 weeks of FPD consumption in our current study, which a variety of factors might explain.

In contrast, the clearance rate of Hp was slightly lower after 8 weeks of FPD consumption in our current study, and a variety of factors might explain.
Reply: Revised as suggested.

Page 11

Participants' diets were not restricted during the study period, and frequent re-infections were possible.

The participant diets were not restricted during the study period, and frequent re-infections were possible.
Reply: Revised as suggested.

Page 12

Limitations of the study, the vast majority of the participants were asymptomatic, and some of them lacked knowledge and awareness of Hp infection, which increased the difficulty in project management and resulted in poor compliance.

As to other limitations of the study, the vast majority of the participants were asymptomatic, and some of them lacked knowledge and awareness of Hp infection, which increased the difficulty in project management and resulted in poor compliance.
Reply: Revised as suggested.

Page 12

No control group with standard practice to fully compare intervention effectiveness. Follow up limited to 8 weeks.

No control group with standard practice was organized to fully compare intervention effectiveness. The follow up period was limited to 8 weeks.

Reply: Revised as suggested.

Reviewer B

I have carefully read the submission entitled "A fucoidan plant drink reduces Helicobacter pylori load in the stomach: a real-world study". The authors tried to make research on the administration of a fucoidan plant drink named Lewuyou (Chinese product) to 122 patients infected with Helicobacter pylori for a period of 4 weeks and 8 weeks.
The authors will make revisions, corrections and clarifications in order to improve the quality of the manuscript as cited below:

Abstract

-Lack of perspectives and recommendations in the abstract.

Reply: To maintain conciseness in the abstract, we did not include the content regarding perspectives and recommendations, which are instead elaborated upon in the Discussion section of the article.

-Add the utilized method « Urea Breath Test »

Reply: The Urea Breath Test (UBT) is mentioned in the abstract (line 58), and detailed information regarding the diagnosis of Hp infection and efficacy is provided in the Methods section of the main text.

-Keywords: Add: Real world, eradication or reduction.

Reply: Revised as suggested.

Introduction

-The authors didn't state the studies on alternative therapies for Hp eradication.

Reply: The introduction mentioned the significance of exploring alternative therapies for Helicobacter pylori, and the revised manuscript added a reference (Reference 15, the eradication effect of Astragalus on Hp), while the discussion section introduced similar studies on fucoidan's clearance of Hp (Lines 333-339).

-What is the prevalence of this infection in China? is higher in adults or children?

Reply: Based on the data observed in current research, the infection rate of Helicobacter pylori in adults is higher, approximately around 50%, while the infection rate in children is around 30%.

-What is the novelty of this research ?? the objective is not clearly established.
Reply: This study observed the natural-state clearance rate of Helicobacter pylori (Hp) following the consumption of a beverage containing fucoidan.

Methods

The methods used in this research are without references.
Reply: This study is a relatively simple observational study with a straightforward design. While the research design lacks specific references, similar studies are discussed in the later sections of the paper.

-Mention the period of the study in the manuscript.
Reply: The intervention period for the study was 4 weeks/8 weeks, as described in lines 178-179 of the manuscript.

-Mention the severity of the infection in the patients (history of the disease)? type of pathologies associated to this bacterium? type of gastric disease (Hp).
Reply: The study mainly observed individuals from the health check-up population, most of whom were asymptomatic, thus there was no stratification.

-These 7 centers are hospitals, clinics ...etc.?
Reply: The 7 study centers are health check-up centers affiliated to hospital-based or independent healthcare facilities.

-The patients are hospitalized? or admitted for consultation?
Reply: The study participants were not hospitalized patients but individuals identified with Hp infection during routine health check-ups.

-Mention the consent of the patients (ethical approval or statement)!
Reply: The intervention product (Lewuyou®) is categorized as a "food product" and the safety considerations for food and pharmaceuticals differ. In China, there is currently no requirement for ethical approval for the use of "food product" in research. But, all research subjects will be informed of the intervention drink, the ingredients of the intervention drink, and the type of drink before enrollment. All participants agreed to take the intervention beverage daily as per the study requirements.

-Add the questionnaire used for the patients (age, sex…).
Reply: The age and gender of the study participants who completed the research are described in the Results section (lines 243-244).

-Mention the source of Lewuyou (Manufacturer)? and the price of this product (economic interest)?
Reply: The Acknowledgements mentioned that the product manufacturer provided research products, but did not provide funding for research in other areas. The market price of the product is 298 yuan/bottle, but the actual sales price is significantly lower than this (purchase gift activity). Currently, the product is mainly used as a gift in hospitals.

-Lewuyou is composed by a lot of products which have Helicobacter pylori activities (green tea, broccoli, radish, Lycium ruthenium… so the effect is not due to Fucoidan only; it's a synergetic effect between all these constituents??? Also, the concentration of Fucoidan (200 mg)? and its side effects (reference)?

Reply: Lewuyou contains various ingredients, but based on current research, the component primarily associated with Hp clearance in the product is fucoidan. Fucoidan is a food extract, and the amount added to Lewuyou is 200 mg per bottle. According to existing studies, this dosage has shown no side effects, and none of our study participants reported any discomfort.

-The frequency of use of this product (4 weeks, 8weeks) is documented?? 8 weeks is sufficient to get best results? in your study, only 41 patients (total number of the patients) are eligible for this study (4 and 8 weeks)? the samples are heterogenous (4 weeks (87), 8 weeks (41)!

Reply: We did not keep a daily record of the participants' consumption, but we conducted oral follow-ups (via phone or WeChat). The taste of Lewuyou is pleasant, and there were no reports of refusal to consume it. All distributed intervention products were consumed, although there may have been occasional deviations in the timing, not strictly adhering to the recommended twice-daily（every morning and evening）.

A total of 122 participants were enrolled in the study. Eighty-five participants completed the 4-week intervention, and among these Eighty-five participants, 41 participants completed the 8-week intervention (lines 243-245).

Line 81: Considerably (C in capital letters).

Reply: Revised as suggested.

Line 89, 295: The reference should be in the end of the sentence.

Reply: Revised as suggested.

Line 90-92: Replace the word « to reduce » by « to prevent ».

Reply: I'm sorry, I couldn't find the word « to reduce » in lines 90-92 of the text or the surrounding context.

Line 190: A sentence without a verb?

Reply: The sentence 'Hp eradication and clearance rates at weeks 4 and 8' represents an observation metric rather than a complete sentence. This part of the content describes the primary observation metrics of this study and, as such, is not a complete sentence."

Results and Discussion

-Discussion of the results is very poor (frequency of use, concentration, other products (drinks, tablets….), period of study, real world studies on other products, in different countries…etc.) Your discussion is mainly on Fucoidan pure and its antibacterial activity (invitro)!

Reply: The frequency of intervention food usage and the content of key ingredients were mentioned in the research methodology. It was administered twice a day (line 178), and the content of the effective ingredient was 200mg per bottle (line 131). The patient recruitment for the study took place from October 2020 to July 2021 (mentioned in the abstract of the original manuscript), and the intervention duration was 4 weeks and/or 8 weeks. Due to the predominant

focus of studies on the fucoidan's eradication effect on Helicobacter pylori being in vitro antibacterial activity and animal research, the discussion section of the paper contains more content related to this aspect. Similar real-world studies that resemble this paper were also mentioned (lines 335-339).

-Conclusion is very short and incomplete!!!!
Reply: Because this study is a relatively simple real-world study, the original intention of the research was merely to understand the natural clearance ability of plant beverages containing fucoidan on Helicobacter pylori. Therefore, the obtained research results are relatively straightforward, and future studies will build upon this foundation to conduct more comprehensive observations in various aspects.

-Rewrite and reformulate the discussion and conclusion.
Reply: The current discussion and conclusion section has been collaboratively written by several authors, and it has undergone multiple revisions from the final draft to the submission stage, suggestions from several relevant professionals have also been consulted. Could you please provide more specific details regarding your modification suggestions? We will then proceed with further revisions based on your recommendations. Thanks.

Acknowledgments
-Add Acknowledgments for patients and health personnel (7 centers).
Reply: The Acknowledgments for patients and health personnel has been added to the Acknowledgments content.

References
-Reference 6: Repetition of 2020!
Reply: Revised as suggested.

-All scientific names of plants and bacteria should be written in italic form.
Reply: Revised as suggested.

-Helicobacter pylori … « p » should be written in small letters.
Reply: Revised as suggested.

-Standardize the writing of the references; the initials of the words sometimes are in capital letters.
Reply: Revised as suggested.

Reviewer C

The analysis I carried out of the manuscript indicates that the vegetable drink fucoidan, although considered a food product, has the property of reducing the rate of Helicobacter pylori (Hp) infection. However, the dose of fucoidan in the product is 200 mg, in a mixture of many other products. If the objective is to evaluate a possible effect on Hp, the composition of

fucoidan in the product must be changed and evaluate possible interactions of the polysaccharide with other ingredients of the Lewuyou product. The analysis performed was interesting and as described in the conclusions: " More research is required to determine the long-term eradication rates, impact on Hp associated diseases and random controlled trials comparing with current standard therapy." The reevaluation of conduct with Human Beings must be careful to avoid nuances such as those reported by the authors in study limitations: "Limitations of the study, the vast majority of the participants were asymptomatic, and some of them lacked knowledge and awareness of Hp infection, which increased the difficulty in project management and resulted in poor compliance. In addition, the custom of eating together among Chinese populations increases the risk of Hp persistence or infection. As a result, the dropout rate was high during the implementation of our study. No control group with standard practice to fully compare intervention effectiveness. Follow up limited to 8 weeks."

My biggest observation concerns the fact that I have not seen any prior analysis from an Ethics Committee that has approved this work with Human Beings. I suggest that the Ethics Committee approval protocol be included. Regarding work, few details need to be corrected.

Reply: Regarding the issue of Ethics Committee, I would like to explain: The intervention product (Lewuyou®) is categorized as a "food product" and the safety considerations for food and pharmaceuticals differ. In China, there is currently no requirement for ethical approval for the use of "food product" in research. But, all research subjects will be informed of the intervention drink, the ingredients of the intervention drink, and the type of drink before enrollment. These have been added to the footnote section of the article.

Please also refer to the comments marked in the PDF file.

Reply: comments marked in the PDF file has been revised as suggested. Thanks.

Reviewer D

Title is suitable.

Text is clear.

Abstract is adequate.

Introduction gives enough information to support the aim of the study, which is clearly stated.

Material and Methods Section is adequate.

Results: experiments are well controlled. Figures are clear.

List of references is in correct format.

Discussion is adequate.

Reply: Thank you for your appreciation of this article.