Fucoidan from *Sargassum Hemiphyllum* Inhibits Infection and Inflammation of *Helicobacter Pylori*

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Research Article

Keywords: Helicobacter pylori, Sargassum hemiphyllum, Anti-adhesion, Anti-bacterial, Anti-inflammation

Posted Date: August 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-717424/v1

Version of Record: A version of this preprint was published at Scientific Reports on January 10th, 2022.
See the published version at https://doi.org/10.1038/s41598-021-04151-5.

Abstract

When infected by *Helicobacter pylori*, it often causes gastritis, gastric ulcer, or gastric cancer. Antibiotics are used to treat *H. pylori* infection, as they inhibit or kill *H. pylori* often ex-tending to reduce the incidences of gastric adenoma and cancer. However, *H. pylori* has developed drug resistance to many clinically used antibiotics over the years, thereby providing no warranty of successful treatment whenever *H. pylori* infection befalls. We report here that fucoidan from *Sargassumhemiphyllum* can effectively reduce infection of *H. pylori* without development of drug resistance. Fucoidan demonstrated a strong anti-inflammation activity in RAW264.7 cell model. Using AGS cell model, fucoidan decreased *H. pylori* adhesion to host cells and thus reduced its infection rate, especially in post-treatment where the infection rate was reduced to 40%. Mechanistically, fucoidan intervenes the proper functions of adhesion molecules BabA and AlpA of *H. pylori*. Moreover, fucoidan is able to significantly lower the total count of *H. pylori* and the levels of IL-6 and TNF-α *in vivo*. Added together, these convergent results suggest that fucoidan is an effective agent in a position to protect stomach from *H. pylori* infection by reducing its total count and induced inflammation.

1. Introduction

It has been well documented that Helicobacter pylori is the causative agent for major gastric associated diseases. The virulence factor CagA of the pathogen has been characterized abnormally activating nuclear factor kappa-B (NF-κB) so as to upregulate production of the inflammation factor interleukin-8 (IL-8) leading to gastritis. If it turns to a chronic condition, gastritis could morph to gastric ulcer, which could end up with gastric cancer[1].

It was estimated about 50% of world population suffer *H. pylori* associated chronic gastritis. Some countries in East Asia including Taiwan are at the top of the rank[2]. In view of public health and wellbeing, how to prevent infection of *H. pylori* from occur-ring and how to effectively treat these diseases are two big concerns. The mainstream treatment of *H. pylori* infection remains the use of antibiotics, which usually combines two types of antibiotics in conjunction with a proton pump inhibitor[3]. Because of extensive and/or abusive use of antibiotics, some strains of *H. pylori* have developed drug resistance, which often makes the treatment a failure[4]. To find more effective antibiotics or substances that can stop or be conducive to the treatment of the *H. pylori* associated chronic gastritis becomes growing interests in the biomedical community.

Seaweeds are known with copious and diverse biological activities, such as an-ti-cancer, anti-virus, anti-bacteria and adjustment of immunity. Brown seaweed stands head and shoulder above the rest. For example, fucoidan, one of key components out of water extracts of brown seaweeds, has been demonstrated with strong anti-cancer[5–7], anti-virus[8], and anti-pathogen adhesion/infection activities[9]. Taiwan is blessed as she is surrounded by seas, where brown seaweeds are abundant, particularly *Sargassum hemiphyllum*.

In this study, we utilized fucoidan from *Sargassum hemiphyllum* to investigate their biomedical effects on *H. pylori* infection *in vitro* and *in vivo*. First, we chemically analyzed the sulfate and total sugar contents of fucoidan. Next, fucoidan was subjected to anti-inflammation and anti-adhesion evaluations against *H. pylori* infection using mouse macrophage (RAW264.7) cell and gastric adenocarcinoma cell (AGS) model systems. Finally, we performed animal experiments, where the fucoidan were gauged with their effects on prevention and treatment of *H. pylori*-infected BALB/c mice. Overall, our results demonstrated a desired protection effect on *H. pylori* infection as well as mitigation of inflammation induced by *H. pylori*.

2. Results

2.1 Yields and compositions of the fucoidan from Sargassum hemiphyllum

Sargassum hemiphyllum was extracted with hot water, by which the yield of fucoidan was estimated to be 13.5%. The molecular weight in GPC analysis showed that the retention time of fucoidan (9.84 min) was similar to that of the retention time of fucoidan purchased from sigma (CAS: 9072-19-9), the molecular weight is 440kDa[10] (Fig. 1). Fucoidan is composed of mainly sulfated polysaccharides, in which the total sugar and sulfate group contents were determined to be 321 mg/g and 12.87%, respectively. Its total phenolic content and protein contents were 18.59% and 20.60%, respectively. The monosaccharide components were 51.2 % fucose, 16.2 % hexuronic acid, 15.8 % galactose, 12.2 % glucose, and 4.6 % N-acetylgalactosamine (Table. 1).

Table 1
The yield, total sugar, sulfate group contents, and monosaccharide components of fucoidan.

Yield (%)	**13.5**	
Molecular weight (kDa)	440	
Total sugar content (mg/g)	321.17 ± 4.58	
Sulfate group content (%)	12.87 ± 1.61	
Total phenolic content (μg/mg)	18.59 ± 1.40	
Protein content (%)	20.60 ± 0.04	
The monosaccharide compositions of polysaccharide	Fucose	51.2 %
	Hexuronic acid	16.2 %
	Galactose	15.8 %
	Glucose	12.2 %
	N-Acetylgalactosamine	4.6 %

2.2 The effect of fucoidan on NO (nitric oxide) production in RAW264.7 cell

Next, we evaluated fucoidan toxicity using the RAW264.7 cell, in which the con-centration that didn't bring about apparent cytotoxicity on RAW264.7 was determined, that is, the concentration of fucoidan up to 1000 μg/mL, and fucoidan shows no cytotoxicity (Fig. 2a). To probe fucoidan on NO inhibition, LPS was added into RAW264.7 cells to induce production of NO, where the NO production in the group added with LPS was set as 100%. In contrast, the LPS-induced NO production was measured for samples added with a series of different concentrations of fucoidan in RAW264.7 cells. The results showed that the concentration of NO is inversely proportional to the quantity of fucoidan added in cells in a dose-dependent manner, where the concentration at 250 μg/mL reaches a significant difference (Fig. 2b).

2.3 Inhibition of H. pylori infection on AGS cells by fucoidan

We then came to examine the inhibitory activity of fucoidan upon *H. pylori* infection. The infection ratio of *H. pylori* to cells was set at 100:1 (Multiplicity of infection, MOI = 100). Our examination included three testing groups: Post-treatment, Pre-treatment, and Co-treatment. The bacteria-challenge experiments showed that fucoidan, in general, is effective against *H. pylori* infection in a dose-dependent manner in the Post-treatment group. Given the highest concentration, 2000 μg/mL, the infection rate is decreased down to 60% in the Post-treatment group, while it shows no inhibitory activity in the other two groups (Fig. 3), suggesting that fucoidan interacts with *H. pylori* directly.

2.4 The activity and mechanism of fucoidan in reducing H. pylori infection

Given that fucoidan effects on the Post-treatment group against *H. pylori* infection, we hypothesized that the effectiveness of fucoidan is attributed to the compromised adhesion of *H. pylori* to host cells. To probe the mechanism behind this phenomenon, AGS cells infected by *H. pylori* were collected and subjected to RT-PCR analysis. The analysis showed that the expression of 16s rRNA was significantly decreased in the cells added with fucoidan (Fig. 4a). Because AGS cells infected by *H. pylori* generally trigger release of pro-inflammatory cytokine IL-8, the reduced production of IL-8 in the bacteria-infected AGS cells is thus ascribed to the treatment of fucoidan (Fig. 4b). The outer membrane proteins (OMPs) AlpA and BabA of *H. pylori* have been characterized as adhesins or adherence-associated proteins. Their participation in the infection of *H. pylori* on AGS cells was therefore analyzed. As expected, fucoidan showed a significant inhibitory effect on the expression of AlpA and BabA in *H. pylori* (Fig. 4c, d). fucoidan is thereby concluded an agent that is in a position to reduce the adhesion ability of *H. pylori* to host cells as well as in a position to inhibit induction of *H. pylori*-associated inflammation responses in host cells.

2.5 The effect of fucoidan on the total count of H. pylori in BALB/c mice

With the promising *in vitro* outcomes, we further evaluated the treatment efficacy of fucoidan on *H. pylori*-infected mice. Our initial assay showed that *H. pylori* mainly masses in the stomach of infected mice, which agrees with empirical results. In terms of *H. pylori* count, both pre-treatment group and post-treatment group (Pre- and Post-Fu groups) showed significantly lower than that of control; the Post-Fu group relatively outperformed the Pre-Fu group. 16s rRNA of *H. pylori* is indicative of its expression; as expected the infected group had highest level of 16s rRNA. By contrast, Post-treatment seems to get an upper hand over Pre-treatment in reducing the total count of *H. pylori* in mice (Fig. 5).

2.6 Histological changes and eosinophils infiltration in gastric tissues of infected mice

Immune cells infiltration as a result of inflammatory responses is closely related to infection of microorganisms. H&E staining is often used to examine whether tissues have experienced immune-cell infiltration. Figure 6 shows that muscularis mucosae of the infected group had undergone noticeable cell infiltration as manifested by irregular stomach mucosa layers and a large number of gathered immune cells. In contrast, the lesions in the fucoidan treated groups seems improved. By means of Giemsa staining (Fig. 7), we found that many eosinophils manifested in brick-red particles in the infected group in agreement with that of H&E staining. Given that the fucoidan treated groups showed significantly diminished eosinophils infiltration, we concluded that fucoidan can regulate immune cells by ameliorating some adverse symptoms upon *H. pylori* infection, for example, eosinophils infiltration.

2.7 Fucoidan reduced pro-inflammatory cytokines in mouse stomach

It has been well known that Th2 cells produce IL-4 and IL-5. To understand whether the fucoidan-modulated-immunity in the *H. pylori*-infected mice follows the Th2 path, we measured the expression levels of IL-4 and IL-5 (Fig. 8a, b). We found that the expression level of IL-4 had no significant difference amid the testing groups, while the expression level of IL-5 is positively correlated to the severity of eosinophils infiltration in the testing groups. Oral administration of fucoidan showed best reduction for the expression of IL-5. On the other hand, IL-6, IL-1β, and TNF-α are representative pro-inflammatory cytokines. The expression levels of IL-6, IL-1β, and TNF-α were increased significantly in the BALB/c mice that were infected with *H. pylori* for two weeks. In contrast, the expression levels of IL-6 and TNF-α were decreased in the infected mice treated with fucoidan; interestingly, only was IL-1β reduced significantly in the Post-Fu group (Fig. 8c, d, e).

3. Discussion

It has been well documented that seaweed *Sargassum hemiphyllum* contains rich polysaccharides, particularly, fucoidan that features multiple sulfate modifications on its polysaccharide chain. In this study, we demonstrated that fucoidan derived from *Sargassum hemiphyllum* is able to modulate the production of NO *in vitro* and *in vivo* in agreement with those of previous reports where fucoidan was praised with desired anti-inflammatory activities[11,12]. Reports also showed that fucoidan can block

functions of selectins, which is closely related to modulations of inflammatory cells in tis-sues as manifested by infiltration and inflammation when the immune system is com-promised.

Sulfated polysaccharides re-attract much attention lately because some sulfated polysaccharides were reported able to interfere adhesion of *H. pylori* to macrophages or other host cells[13–16], where *H. pylori* literally interacts with host cells through sulfate-polysaccharide mediated receptors for binding on cell surface. Fucoidan from *Cladosiphon okamuranus* was reported able to lower the total count of *H. pylori* in human stomach[17, 18]. We were therefore tempted to explore the protective function and effective mechanism of fucoidan from *Sargassum hemiphyllum* upon *H. pylori* infection using anti-adhesion assay.

H. pylori is known to express adhesin BabA[19], which takes part in binding with host receptors and thereby enhancing infection of *H. pylori* T4SS resulting in serious inflammation and eventual tumorigenesis[20]. AlpA is a lipoprotein, which exists on the cell membrane of *H. pylori*. AlpA is another important adhesin that adheres to human stomach tissues. When overexpressed, AlpA invokes strong oxidation pressure. On top of that, AlpA induces pro-inflammatory cytokines severely damaging stomach tissues[21]. In contrast, fucoidan is able to associate with BabA and AlpA thereby prohibiting *H. pylori* adhesion to host cells. Additionally, fucoidan contains a high content of fucose, which is capable of interacting with *H. pylori* thereby synergistically counteracting the adhesion of *H. pylori* to gastric epithelial cells.

One has known that hosts respond to the infection of *H. pylori* by secreting a considerable amount of IL-8. IL-8 is an important immunological effector, which acts as an index of recurrent tendency of cancer, because IL-8 could stimulate angiogenesis, inflammation, and proliferation/translocation of epidermal cells. Given a high level of IL-8 in body, the risk of contracting cancer is increased substantially. The onset of tis-sue lesions is often resulted from the recalcitrant and hard-to-remove *H. pylori* infection and there are further complications with persistent secretion of IL-8. In this study, we identified that fucoidan significantly reduced the count of *H. pylori* and the level of IL-8 in the Post-Fu group.

It has been reported that the higher the fucoidan level the better the efficiency in inhibiting infection and inflammation of *H. pylori* infection[22]. To extend our *in vitro* experiment to *in vivo* one, we adopted pre-treatment and post-treatment groups with-out the co-treatment group, because the samples needed to be mixed with *H. pylori* whereby the condition will not be consistent with that of the other two groups. As expected, fucoidan exerts its effects significantly reducing the count of *H. pylori* in the stomach of mice particularly in the group of Post-Fu. The effect of Post-Fu is relatively better than that of Pre-Fu, while the latter is still outperformed the control (infected group).

IL-6, IL-1β, and TNF-α are pro-inflammatory cytokines. They all are upregulated when infected with *H. pylori*. Among them, IL-6 activates the STAT3 pathway, which is correlated to *H. pylori*-associated gastritis and gastric cancer[23]. Similarly, *H. pylori* infection increases expression of IL-1β, which results in decreased secretion of gastric acid agreeing with the report that mouse developed gastric cancer given increased production of IL-1β [24]. Moreover, a higher *H. pylori* 16s rRNA gene expression in feces was

detected, which is positively correlated with TNF-α activity. High levels of TNF-α normally come with chronic inflammation[24]. While fucoidan did not significantly reduce the level of IL-1β, it significantly decreased the level of IL-6 and TNF-α in mice. Our results demonstrated that fucoidan does carry both anti-inflammation and anti-*H. pylori* activities. Importantly, fucoidan does have the potential to lower the risk of *H. pylori*-associated gastric ulcer or stomach cancer *in vivo*.

Based on our *in vitro* and *in vivo* experiments, fucoidan is demonstrated able to exert its unique biological properties to reduce the risk of *H. pylori* infection as well as the expression of inflammatory effectors effectively. The post-treatment regimen is relatively more efficient than the pre-treatment one in terms of effectiveness *in vivo*. Mechanistically, fucoidan can intervene the adhesion process of *H. pylori* to AGS cells and inhibit the production of NO in Raw 264.7 cells. Despite the biomedical superiority, fucoidan per se is not enough to clear *H. pylori* to complete extent in mice. However, it is more than capable of playing an auxiliary role, such as a medical supplement or a health care product, complementary to formal medical therapeutics.

4. Materials And Methods

4.1 Preparation of the fucoidan

Using a specific ratio mixed the powder of *Sargassum hemiphyllum* (g) and ddH_2O (mL), then heating to 100℃ for one hour. After cooling, adding the specific enzymes reacted for three hours in the shaking incubator, then heating to terminate the reaction of the enzymes. It filtered the extracts to remove the impurities and Centrifuged (5000 x g, 4 ℃,10 min) to collect the supernatant. Finally, fucoidan was acquired by freeze-drying.

4.2 Analytical method of polysaccharide

Molecular weight was measured by gel permeation chromatography (GPC) using the OHpak SB-804 HQ column (Shodex, Tokyo, Japan)[25]. Fucoidan was analyzed by the phenol-sulfuric acid method[26] to identify the total sugar content. Sulfate content was determined by the turbidimetric method[27]; moreover, the protein content and total phenolic content were measured by Bradford assays[28] and Folin–Ciocalteu method[29], respectively. Monosaccharide analysis was determined by the naphthim-idazole (NAIM) saccharide labeling method[30] and used a purchased polysaccharide component assay kit (SugarLighter Corporation, Taiwan).

4.3 Cell culture

AGS (BCRC 60102) cells were provided from National Yang-Ming University (Taipei, Taiwan) and cultured in the RPMI-1640 medium supplement with 10 % fetal bovine serum (FBS), which added the extra essential amino acid, and then cultured in the 37 ℃, 5 % CO_2 atmosphere. RAW264.7 cells (ATCC TIB-71TM) were bought from ATCC (Manassas, USA) and cultured in the DMEM medium supplemented with 10 % FBS in the 37 ℃, 5% CO_2 atmosphere.

4.4 The culture of H. pylori

H. pylori (BCRC15415) was bought from the Food Industry Research and Development Institute Bioresearch Collection and Research Center (Hsinchu, Taiwan). *H. pylori* was cultured under 37℃, microaerophilic for 3–4 days. Then, used 10 % FBS-BHIB and directly scraped the colony from the plate for collection, and then maintained it in TSA-blood plate.

4.5 MTS assay

Cytotoxicity of fucoidan was measured by MTS assay. Cells were incubated in 96 well plates and treated 100 μL different concentrations of fucoidan in a medium containing 2 % FBS in sequence at 37 ℃ for 24 hours. At due time, 20 μL the MTS reagent was added into 96 well plates, which incubated for 1 hour at 37 ℃. Absorbance was measured at 490 nm.

4.6 NO assay

Cultured RAW264.7 cells with 2×10^5 cells / well into 96 well plate. After the cells adhesion, added fucoidan with different concentrations into the well plate, and separately added 1 μg/mL LPS to induced cells producing nitrate for 24 hours. And then, transferred part of the supernatant to the other 96 well plate. After mixed with Griess reagent A and Griess reagent B reacting in the dark, measured the absorption under 550 nm.

4.7 The adhesion test of H. pylori

First, cultured AGS cells (1×10^5 cells/well) into 96-well plate overnight, removed the medium next day, and then washed the plate with PBS twice. After that, proceeded the experiences with three methods:

(1) Pre-treatment: First of all, added different concentrations of fucoidan to re-acted with AGS cells for 2 hours, after that washed with PBS triple times, then added *H. pylori* suspension to infected the AGS cells for 2 hours.

(2) Co-treatment: Cultured different concentrations of fucoidan and *H. pylori* suspension at the same time for 2 hours, then added the mixed medium into the cell, and then cultured for 2 hours.

(3) Post-treatment: Added *H. pylori* suspension to cause the infection for 2 hours at first. Next, washed PBS for triple times, then separately added the fucoidan in distinct concentrations, and then co-cultured for 2 hours.

The multiplicity of infection of three experiments was 100:1 (M.O.I = 100). All the treatment of *H. pylori* suspension and fucoidan was essentially washed with PBS triple times, then reacted in the urease broth for 4 hours, and the absorbance was measured under 560 nm. Calculated the ratio of the infection which was the cells infection by *H. pylori*.

4.8 The experimental design of the infection of H. pylori in vivo

The five-week-old BALB/c mice were purchased from National Laboratory Ani-mal Center (Taipei, Taiwan). All animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan Ocean University (NTOU), and the study conformed to the guidelines of the protocol IACUC-101006 approved by the IACUC ethics committee of NTOU. This study was conducted in accordance with the ARRIVE guidelines. 24 mice were divided into four groups, which were respectively represented Control, Infected, Pre-treatment group (Pre-Fu), and Post-treatment group (Post-Fu), six mice for each group. Before proceeded the treatment, mice were acclimated to the new environment and the diet for one week. Before proceeded with the treatment, mice were acclimated to the new environment and the diet for one week. Mice were challenged by *H. pylori* (1×10^9 CFU/mL) on Day 0 and 2. In Pre-Fu group, before mice were challenged by *H. pylori*, treated 800 mg/kg/day fucoidan to the mice for one week, then continuously treated fucoidan until mice were sacrificed. On the other hand, after mice were challenged by *H. pylori* for one week, mice were treated 800 mg/kg/day fucoidan in Post-Fu group. Control and Infected group fed deionized water. At the end of experience, sacrificed the mice and took of the stomach under the aseptic environment.

4.9 Detection of H. pylori and cytokine RNA expression in AGS cells and mouse gastric tissues

According to the different situations of the experiment. After *H. pylori* infected cells and added fucoidan incubated for 6 hours. Then, washed the cells with PBS, collected the cell pellet to extract RNA. *In vivo*, trimmed the stomach tissue between 25 to 30 mg to extract the total RNA with RNeasy mini kit (QIAGEN, Germantown, USA). The RNA primers were 16s rRNA, BabA, AlpA, IL-8, IL-4, IL-5, IL-6, IL-1β, TNF-α, and IFN-γ, and the primer sequences were used: *H. pylori* 16s rRNA forward 5'-CGATGGATGCTAGTTGTTGGAG-3', *H. pylori* 16s rRNA reverse: 5'- GTCCCCGTCTATTCCTTTGAGTT-3', *H. pylori* BabA forward: 5'- GGTGGGGTTTT-GGAATGTCTTA-3', *H. pylori* BabA reverse: 5'-AAAGAACAGGTGATGGAAGTG-GA-3', *H. pylori* AlpA forward: 5'- GGTAGGCTCTGGGACTTGTG-3', *H. pylori* AlpA reverse: 5'- TGGTGTTCGTGCCGTAGTTA-3', AGS IL-8 forward: 5'- ACA-CYGCGCCAACACAGAAATTA-3', AGS IL-8 reverse: 5'- TTTGCTTGAAGTTTCAC-TGGCATC-3', AGS GAPDH forward: 5'-GCACCGTCAAGGCTGAGAAC-3', AGS GAPDH reverse: 5'- TGGTGAAGACGCCAGTGGA-3', mouse IL-4 forward: 5'- CCAAGGTGCTTCGCATATTT-3', mouse IL-4 reverse: 5'- ATCGAAAAGCCCGAAA-GAGT-3', mouse IL-5 forward: 5'- GTGGGGGTACTGTGGAAATG-3', mouse IL-5 re-verse: 5'- ACCAAGGAACTCTTGCAGGT-3', mouse IL-6 forward: 5'- TCCATCCAG-TTGCCTTCTTG-3', mouse IL-6 reverse: 5'-TTTCTCATTTCCACGATTTCCC-3', mouse IL-1β forward: 5'- CGCAGCAGCACATCAACAAGAGC-3', mouse IL-1β reverse: 5'- TGTCCTCATCCTGGAAGGTCCACG-3', mouse TNF-α forward: 5'- AGCCCCCAC-TCTGACCCCTTTAC-3', mouse TNF-α reverse: 5'- TGTCCCAGCATCTTGTGTTTCT-3', mouse GAPDH forward: 5'- TGCACCACCAACTGCTTAG-3', mouse GAPDH reverse: 5'- GGATGCAGGGATGATGTTC-3'. RT-PCR was set at 95 °C initially for 30s, fol-lowed by 40 cycles of 95 °C for 15 s, 55 °C for 15 s, 72 °C for 45 s. the gene expression level in each sample was normalized using GAPDH.

4.10 Histopathological analysis

After the mice were sacrificed, the stomach tissue was sampled from each mouse. The samples were fixed in 10 % formalin for 24 h at room temperature. The fixed stomach tissue was trimmed into an appropriate size; and paraffin sections were cut at 4 μm. The sections were stained with hematoxylin-eosin (H & E) and Giemsa for observing the presence of inflammatory response in the muscularis mucosae and eosinophil granulocytes in the specimen. Microscopic observations were carried out at 400 x magnifications.

4.11 Statistical analysis

All the data of experiences were expressed as the mean ± SD, and the statistical comparisons were made by Student's t-test. P-values < 0.05 were considered statistically significant, which would mark with " * ".

Declarations

Author Contributions: Yi-Lin Chan, and Chang-Jer Wumeditatedand design this study.Bo-Rui Chen, and Wei-Ming Lianalyze the dataand prepared figures.Bo-Rui Chen and Tsung-Lin Li wrote the main manuscript text. All authors reviewed the manuscript.

Funding: This study was supported by a Grant from the Ministry of Science and Technology, Taiwan (MOST 105-2622-B-019-002-CC2 and MOST 106-2622-B-019-001-CC2).

Conflicts of Interest: The authors declare that they have no competing interests.

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Figures

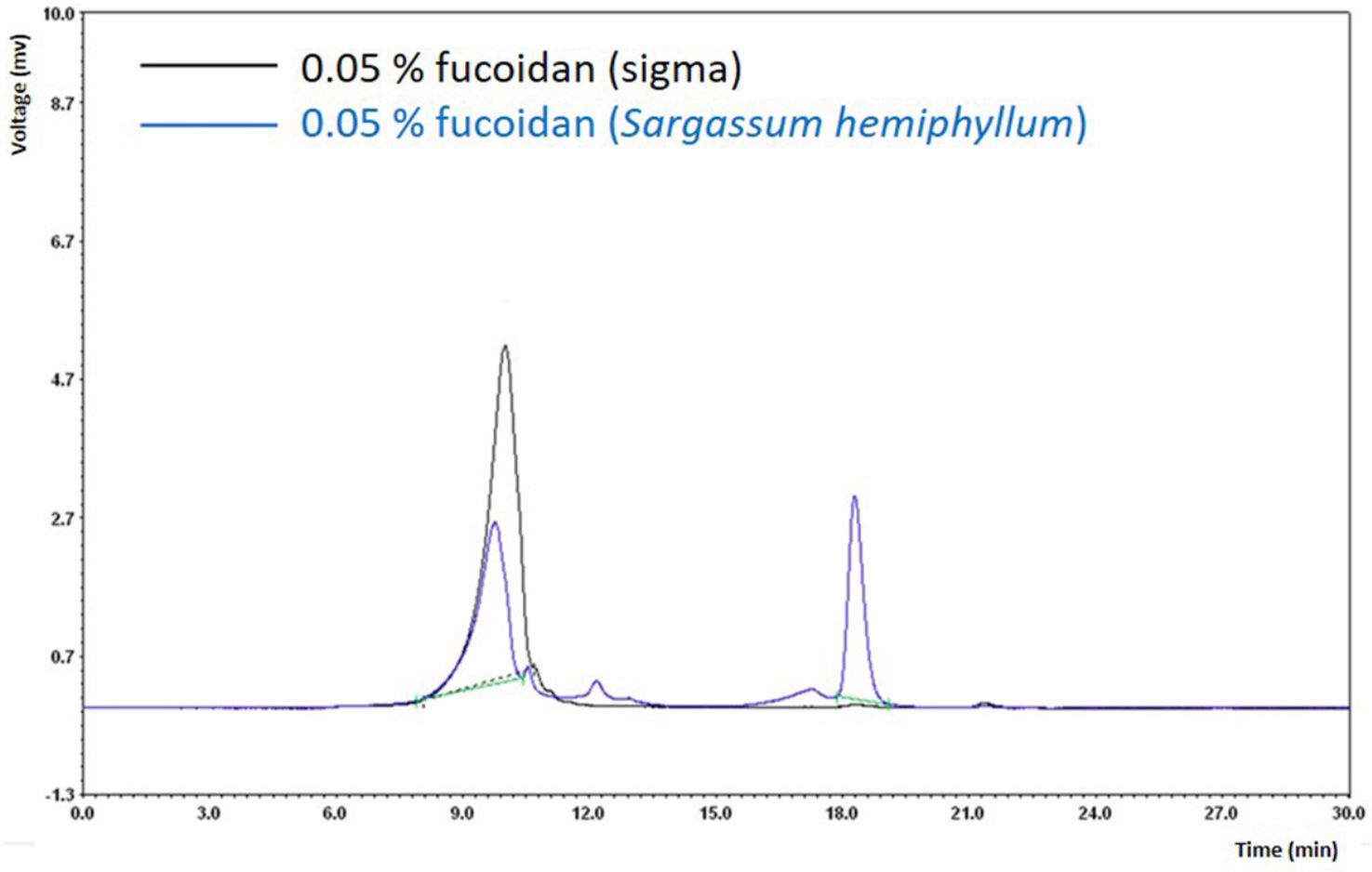

Figure 1

GPC chromatograms of fucoidan from Sargassumhemiphyllum. Column: Ohpak SB-804 HQ, size exclusion column; 8.0 mm ID × 300 mm; column temperature: 35 °C; injection volume: 20 μL; flow rate: 0.8 ml/min; mobile phase: H2O; RI detector (refractive index). Fucoidan purchased from sigma (CAS: 9072-19-9), the molecular weight is 440 kDa.

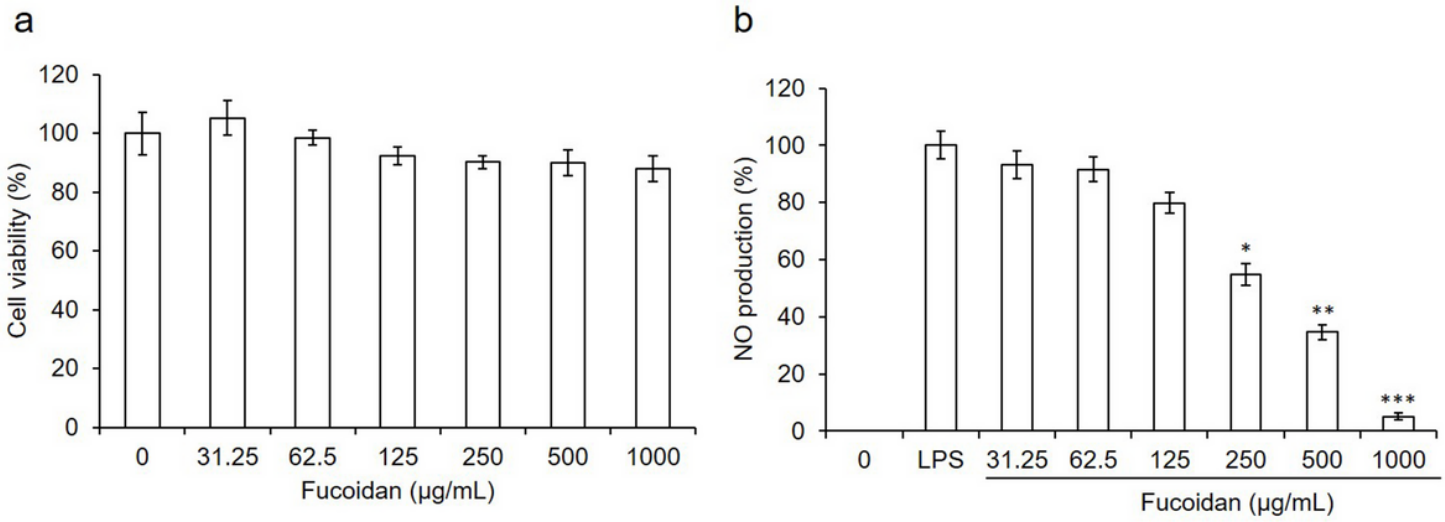

Figure 2

Cytotoxicity of fucoidan on RAW264.7 (a). Inhibitory effect of fucoidan on LPS-induced NO production. RAW264.7 cells were treated with fucoidan in the presence of LPS (1 μg/mL) (b). Triple independent experiments were performed and values were calculated as mean ± SD in each group. Results were statistically analyzed with Student's t-test (*p < 0.05, **p < 0.01, ***p < 0.001 compared with the LPS group without fucoidan).

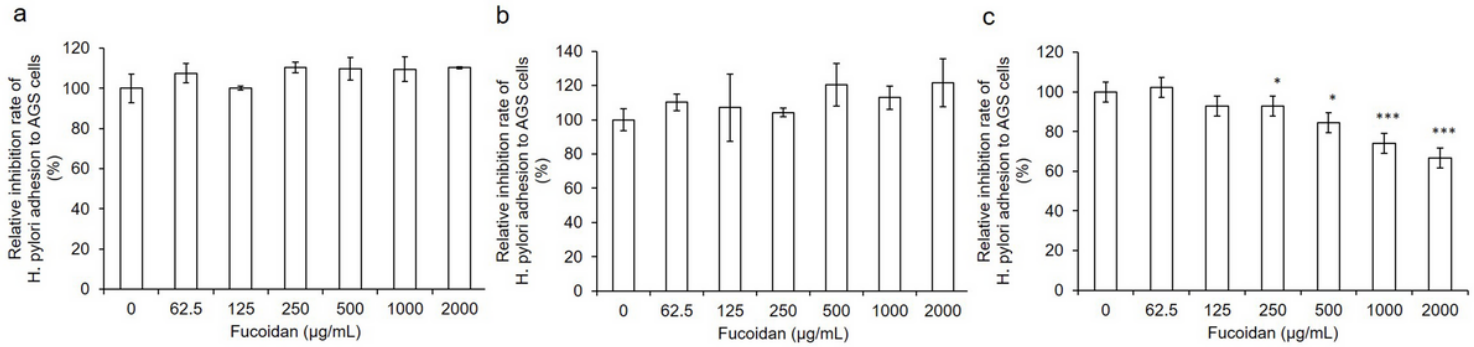

Figure 3

Inhibitory activity of fucoidan on H. pylori-infected AGS cells. The experiment was divided into three groups: Pre-treatment (a), Co-treatment (b) and Post-treatment (c). Triple independent experiments were performed and values were calculated as mean ± SD in each group. Data with different letters were significantly different (*p < 0.05, **p < 0.01, ***p < 0.001 compared with the control group).

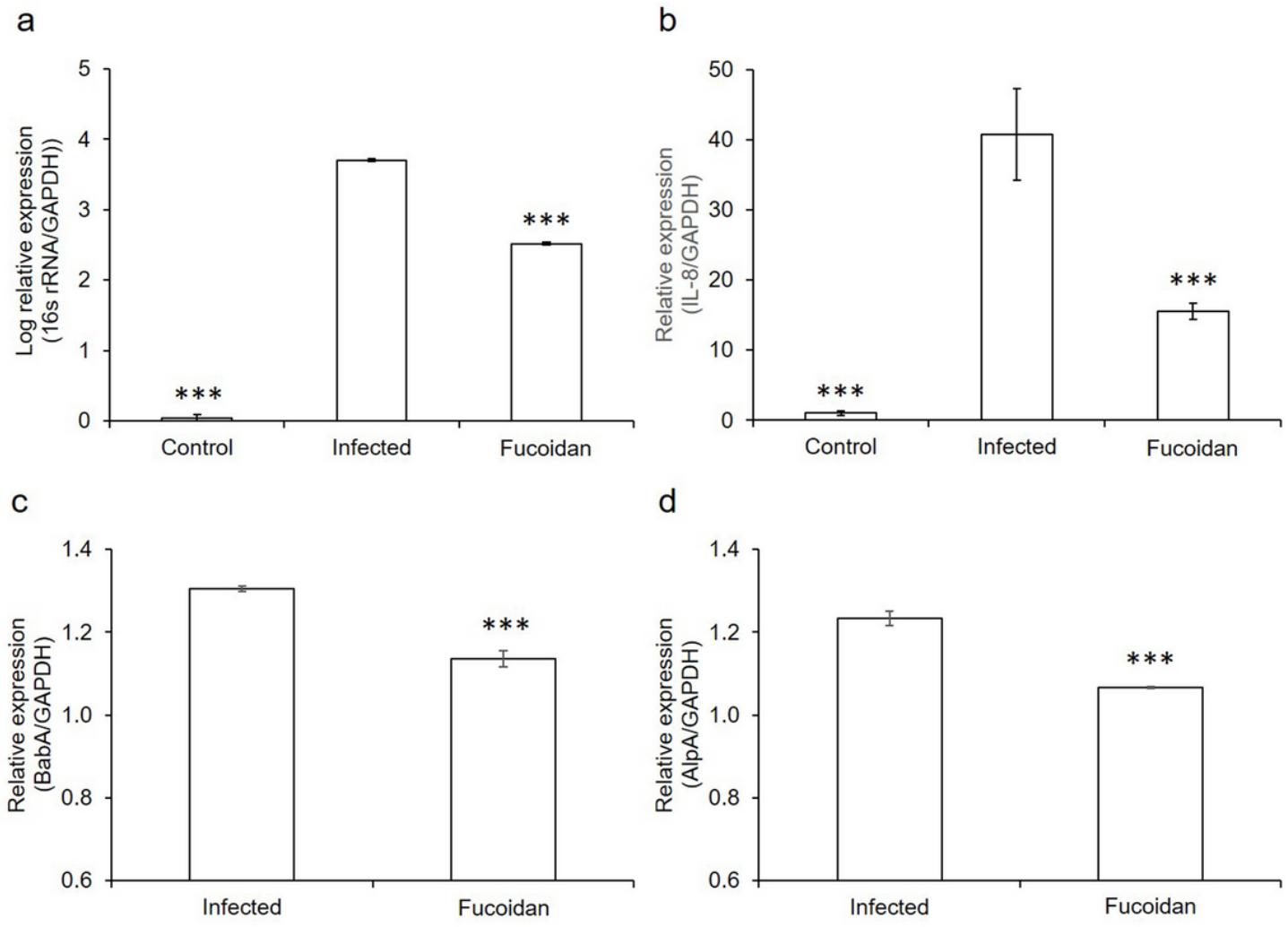

Figure 4

Effects of fucoidan on the mRNA expression of cytokines in H. pylori infected AGS cells. Gene expression levels of 16s rRNA (a) and IL-8 (b) analyzed by RT-PCR; GAPDH acts as a loading control. Gene expression levels of BabA (c) and AlpA (d) analyzed by RT- PCR; 16s rRNA acts as a loading control. Each value was expressed as mean ± SD from triplicate independent experiments. Results were statistically analyzed with Student's t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the infected group).

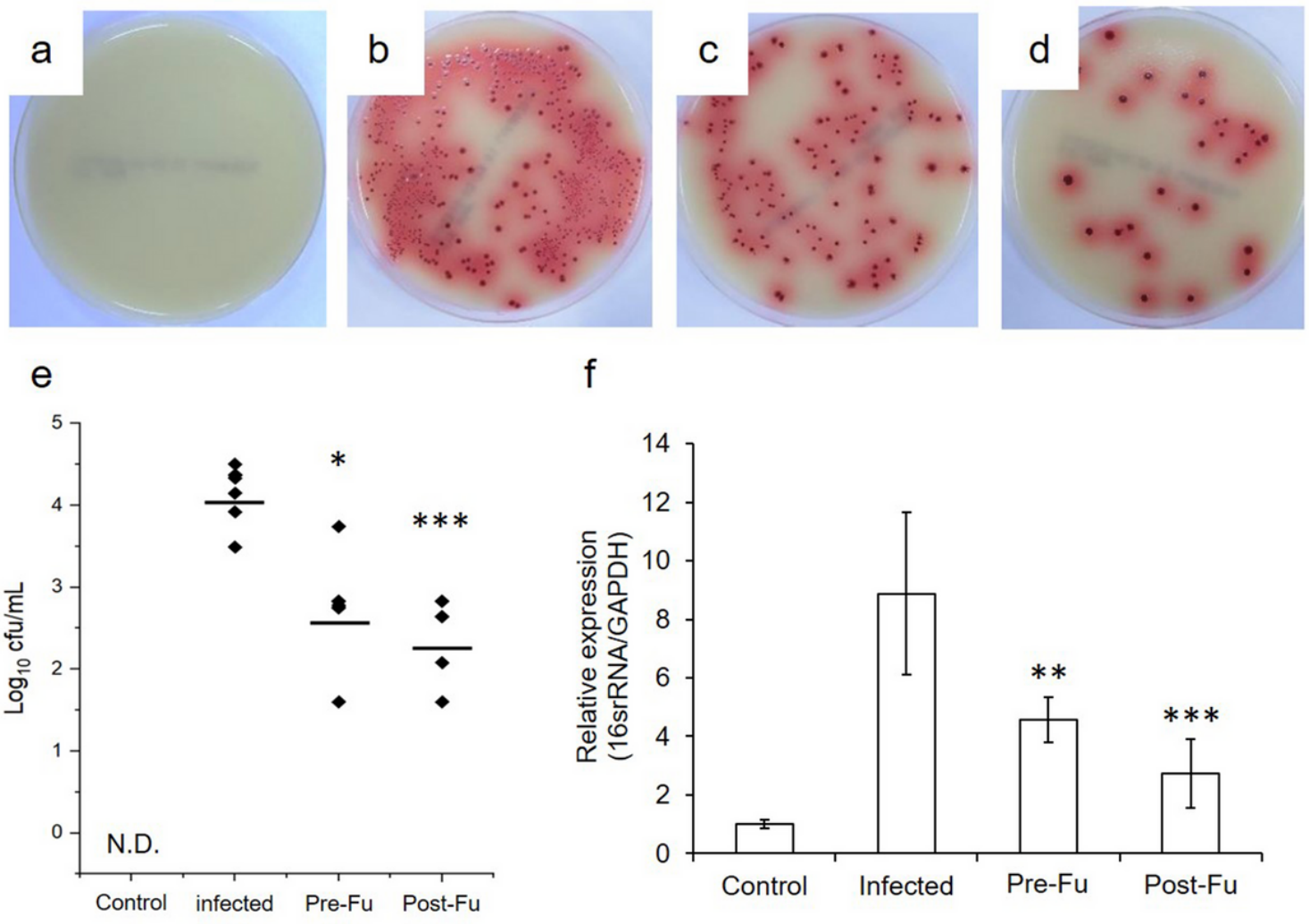

Figure 5

Colony formation analysis where H. pylori (BCRC 15415) was isolated from gastric tissues of infected mice. After 2 weeks' infection, intact stomachs of mice were removed and half-homogenized. The gastric tissues were then streaked onto selective agar plates under a microaerophilic condition at 37℃ for 3-5 days. (a) Control, (b) Infected, (c) Pre-Fu, (d) Pre-Fu, (e) colony count. Gene expression level of 16srRNA(f) analyzed by RT- PCR. GAPDH acts as a loading control. Each value was calculated as mean ± SD for mice in each group (n = 6). Results were statistically analyzed with Student's t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the infected group).

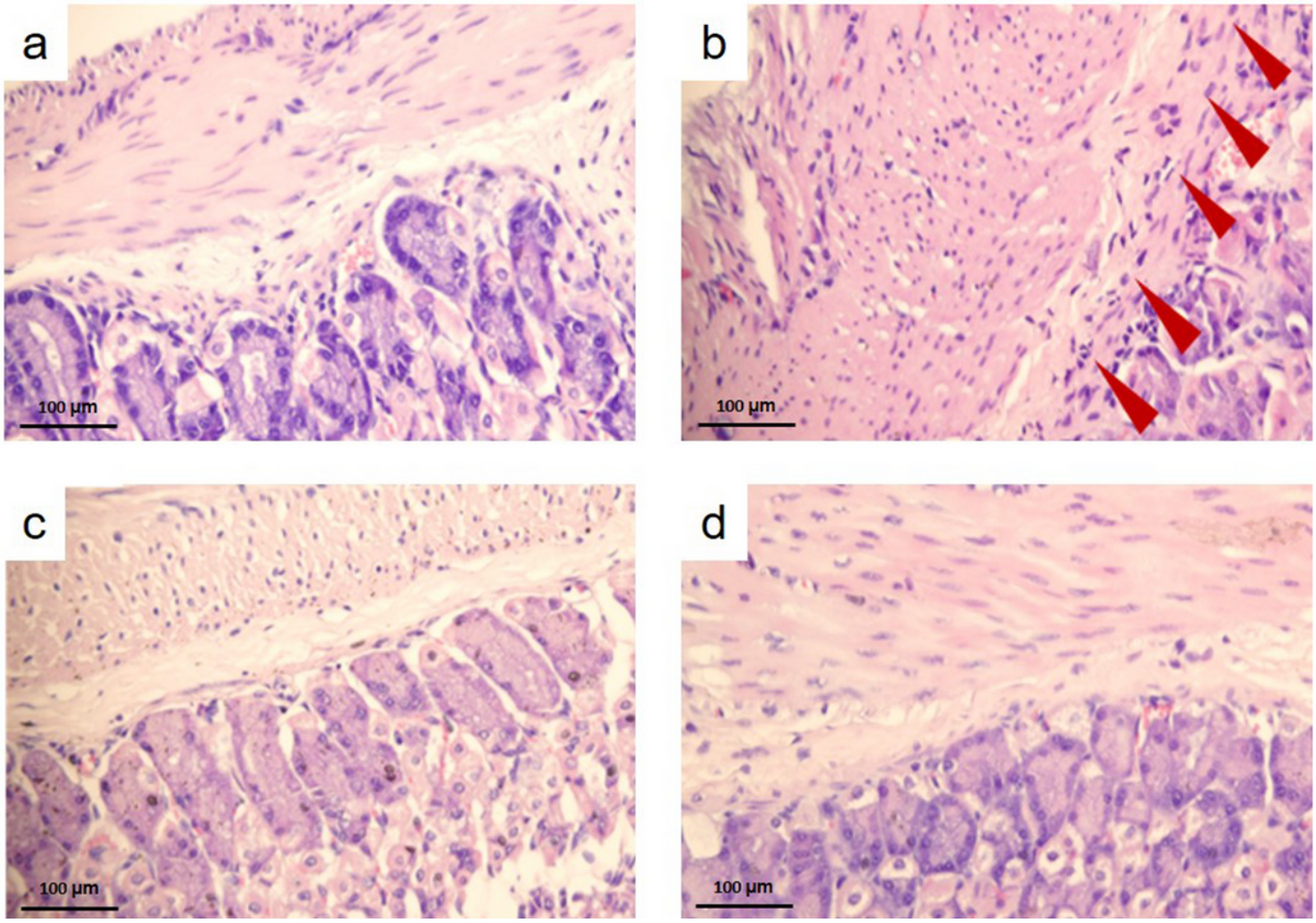

Figure 6

Histopathologic analysis of stomach tissues in H. pylori infected 5-weeks-old BALB/c mice by H&E staining. After 2 weeks' infection, intact stomach tissues were removed from mice. The specimen was embedded in paraffin wax, and the stomachs were stained with H&E to contrast where the gastric inflammation appeared. Red arrows indicate the presence of inflammatory cells in the muscularis mucosae in the specimen. (a) Control, (b) Infected, (c) Pre-Fu, (d) Post-Fu.

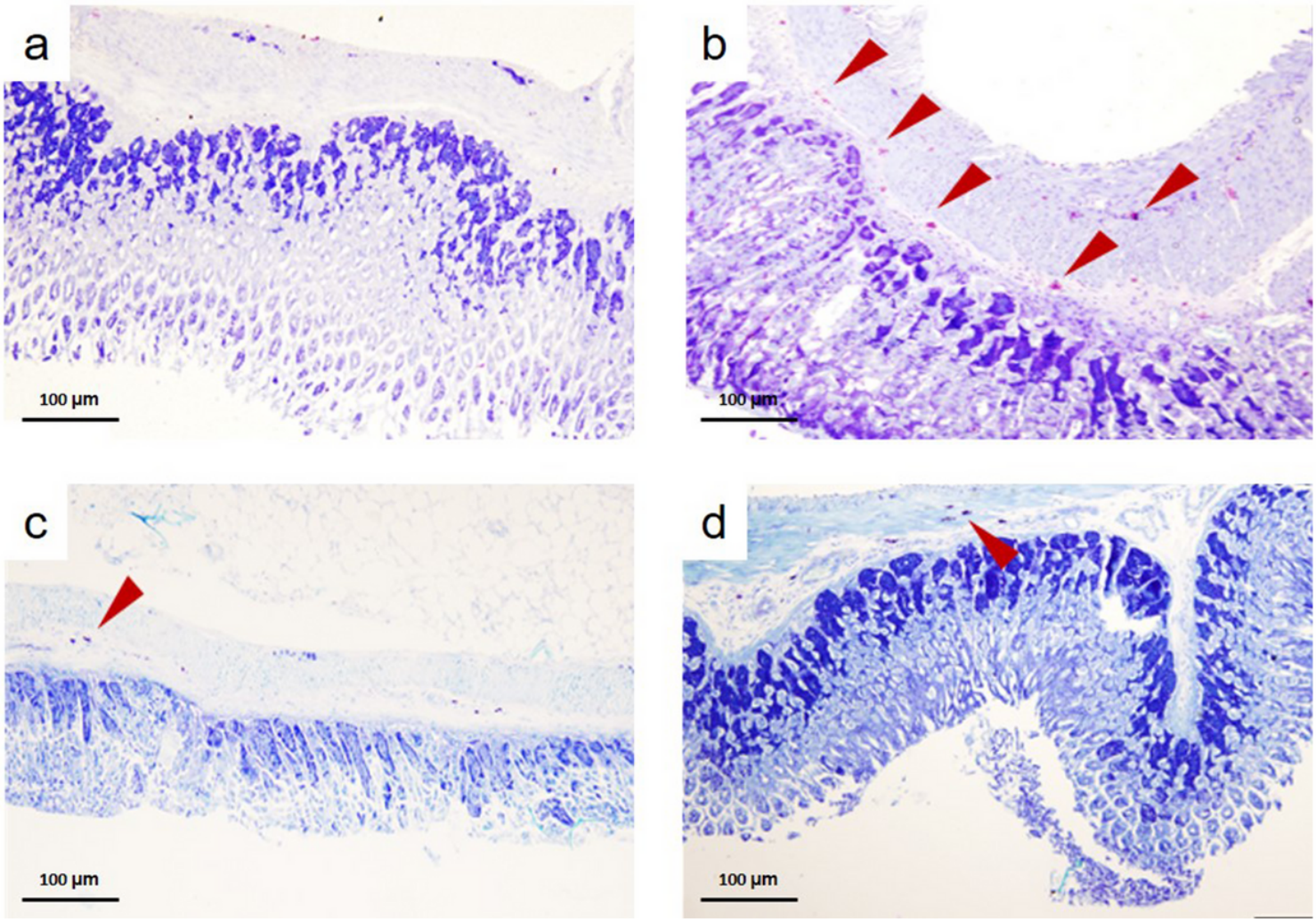

Figure 7

Histopathologic analysis of stomach tissues in H. pylori infected 5-weeks-old BALB/c mice by Giemsa staining. After 2 weeks' infection, intact stomachs were re-moved from mice. The specimen was embedded in paraffin wax, and the stomachs were stained with Giemsa to contrast where the gastric inflammation appeared. Red arrows indicate the presence of eosinophil granulocytes in the specimen. (a) Control, (b) Infected, (c) Pre-Fu, (d) Post-Fu.

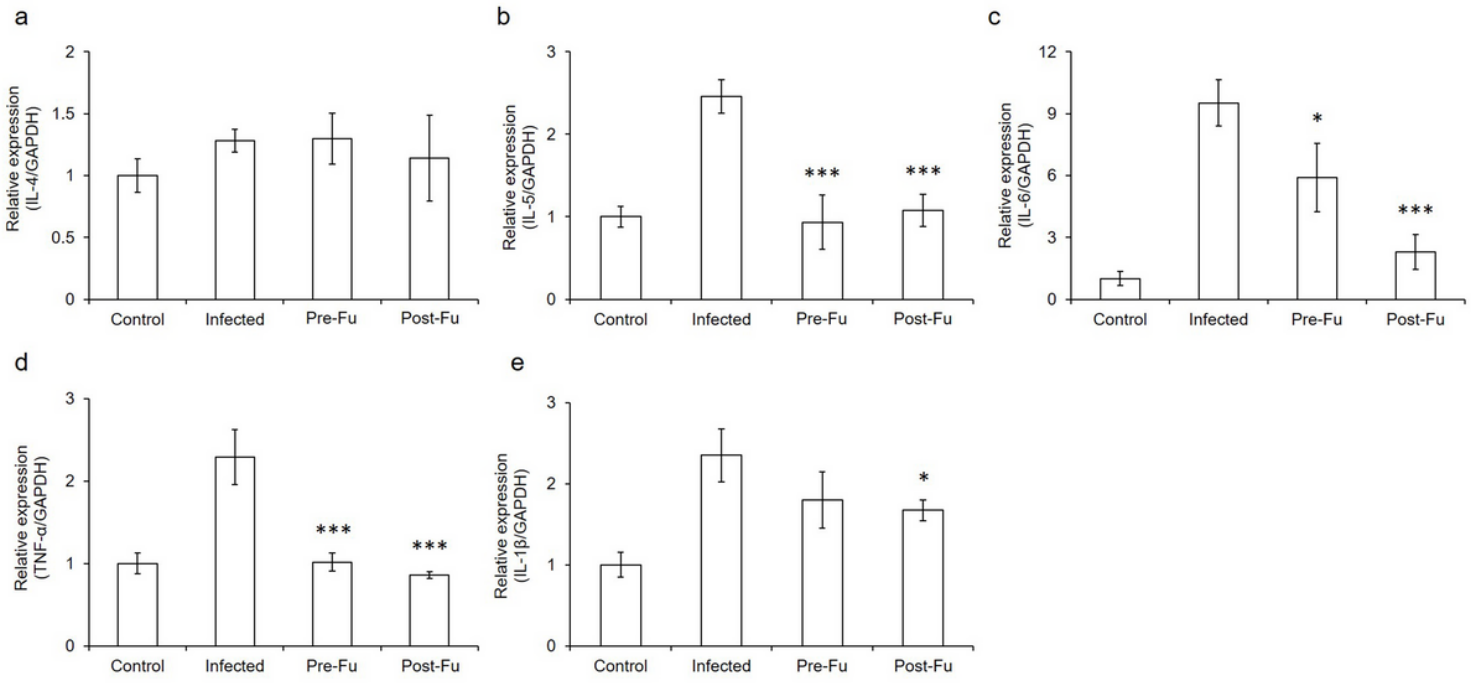

Figure 8

Effects of fucoidan on the expression of IL-4, IL-5, IL-6, TNF-α, and IL-1β in the stomach of H. pylori-infected 5-weeks-old BALB/c mice with/without administration of fucoidan. Mice were infected with H. pylori and were sacrificed after 2 weeks. Gene expression level of IL-4, IL-5, IL-6, TNF-α, and IL-1β was analyzed by RT-PCR. GAPDH acts as a loading control.Each value was calculated as mean ± SD for sample in each group (n = 6). Results were statistically analyzed with Student's t-test (*p < 0.05, **p < 0.01, ***p < 0.001 compared with the infected group).

Supplementary Files

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