



Letter

## Anti-*Helicobacter pylori* activities of FEMY-R7 composed of fucoidan and evening primrose extract in mice and humans

Tae-Su Kim<sup>1</sup>, Ehn-Kyoung Choi<sup>2</sup>, Jihyun Kim<sup>2</sup>, Kyungha Shin<sup>2</sup>, Sung-Pyo Lee<sup>1</sup>,  
Youngjin Choi<sup>2</sup>, Joseph H. Jeon<sup>3</sup>, Yun-Bae Kim<sup>2\*</sup>

<sup>1</sup>Misuba RTech Co., Ltd., Asan, Korea

<sup>2</sup>College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea

<sup>3</sup>Haewon Biotech Inc., Seoul, Korea

**Helicobacter pylori**-eliminating effects of FEMY-R7, composed of fucoidan and evening primrose extract, were investigated in mice and humans. Male C57BL/6 mice were infected with the bacteria by intragastric inoculation ( $1 \times 10^9$  CFU/mouse) 3 times at 2-day intervals, and simultaneously, orally treated twice a day with 10 or 100 mg/kg FEMY-R7 for 2 weeks. In *Campylobacter*-like organism-detection test, FEMY-R7 markedly reduced the urease-positive reactivity. In a clinical study, human subjects, confirmed to be infected with *Helicobacter pylori*, were orally administered twice a day with a capsule containing 150 mg FEMY-R7 for 8 weeks. FEMY-R7 significantly decreased both the Delta over baseline-value in urea breath test and the serum pepsinogens I and II levels. The results indicate that FEMY-R7 not only eliminates *H. pylori* from gastric mucosa of animals and humans, but also improves gastric function.

**Keywords:** *Helicobacter pylori*, FEMY-R7, fucoidan, evening primrose extract, *Campylobacter*-like organism-detection test, urea breath test, pepsinogen

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In peptic ulcers, gastric erosions and ulcers are caused by various factors such as gastric acid over-secretion and retention, mucin layer depletion, blood flow disturbances, and local inflammation [1-4]. There are many ulcer-inducing agents, including non-steroidal anti-inflammatory drugs (NSAID) [5-9], alcohols [7,8,10], stresses [4,7,8], gastric retention [7,8], gastric hypermotility and acetic acid accumulation [8,11-14], and bacterial infection such as *Helicobacter pylori* [1,2,15-17].

*H. pylori* infection is found in gastric ulcer (70%), gastritis (50-60%), and duodenal ulcer (90%) patients. It is well known that *H. pylori* is a key factor for chronic active gastritis as well as development to gastric cancers [18-20]. It is believed that *H. pylori* exacerbates erosions and ulcers via continuous stimulation of gastric secretion and retention [21,22]. Thus, eradication of *H. pylori* is a

key point for ulcer treatment in adults exhibiting a high incidence [1,2,15-17,23].

For both the elimination of *H. pylori* and treatment of gastric ulcers, triple therapies containing proton-pump inhibitors (pantoprazole, omeprazole, lansoprazole, etc) and antibiotics (clarithromycin, metronidazole, amoxicillin, etc) have been recommended [2]. However, it is well known that the antibiotics used for triple therapy display a rapidly-increasing tolerance to *H. pylori* [24]. In spite of relatively-weak potency compared with antibiotics, therefore, natural products without tolerance during repeated administration may contribute to the eradication of the bacteria.

Fucoidan, sulfate polysaccharide complex from *Laminaria japonica* and *Cladosiphon okamuranus*, has been widely used in Oriental medicine. In previous studies, it has

\*Corresponding author: Yun-Bae Kim, College of Veterinary Medicine, Chungbuk National University, 52 Naesudongro (Gaesin-dong), Cheongju, Chungbuk 361-763, Korea  
Tel: +82-43-261-3358; Fax: +82-43-271-3246; E-mail: solar93@cbu.ac.kr

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been demonstrated that fucoidan exerts anti-oxidative, anti-coagulative, and anti-inflammatory activities [25,26]. Accordingly, the beneficial effects of fucoidan on inflammatory diseases, ischemia, and immune dysfunction are attracting investigators' attention [27,28]. Recently, investigators showed that fucoidan inhibited the attachment of *H. pylori* to gastric cells in Mongolian gerbils and in humans [29,30]. Tannins from evening primrose have anti-bacterial activity against *H. pylori*, too [31]. Furthermore, evening primrose extract was found to inhibit bacterial growth *in vitro* and block adhesion and colonization of *H. pylori* in the gastric walls [32]. We also demonstrated that a combinational treatment with fucoidan and evening primrose extract killed *H. pylori* and eliminated the bacteria from the mouse stomachs *in vitro* and *in vivo* [33].

In the present study, we investigated the *H. pylori*-eliminating effects of FEMY-R7, a combinational preparation of fucoidan and evening primrose extract, in mice and in humans infected with *H. pylori* by confirming the presence of bacteria in *Campylobacter*-like organism (CLO)-detection test and urea breath test (UBT), respectively.

FEMY-R7 containing fucoidan and evening primrose seed extract (1:1) was obtained from Misuba RTech Co. (Asan, Korea). Fucoidan was extracted with an acid-hot water extraction method at pH 2.0 and 60°C for 2 hours from *Laminaria japonica* [33]. The extraction supernatant was neutralized with 10 N NaOH and filtered. After precipitation with ethanol, the filtrate was centrifuged and dried. Evening primrose seeds were defatted and extracted with 60% ethanol. The extract was filtered, concentrated, and dried. The 1:1 (v/v) mixture of fucoidan (as *L. japonica* extract) and evening primrose seed extract, named FEMY-R7 [33], was stored at 2°C until use. In Bio-LC analysis, FEMY-R7 was found to contain 7-15% fucose and 0.1-0.4% penta-*O*-galloyl- $\beta$ -D-glucose.

Male C57BL/6 mice (body weights 25-27 g) were procured from Daehan Biolink (Eumseong, Korea), and housed in a room with constant environmental conditions (23±2°C; 55±10% relative humidity; 12-hour light-dark cycle; 150-300 lux brightness). Pellet feed and purified water were available *ad libitum*. All the animal experiments were conducted according to the Standard Operation Procedures (SOP), and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Korea (Approval No. CBNUR-284-11).

After 12-hour fasting, the mice (n=6/group) were

orally inoculated with *H. pylori* SS1 (1×10<sup>9</sup> CFU/1 mL/mouse) 3 times at 2-day intervals, and simultaneously, orally treated twice a day with 10 or 100 mg/kg FEMY-R7 (20 or 200 mg/kg/day) for 2 weeks. Three hours after the final administration, the mice were sacrificed and their gastric mucosa was biopsied for the detection of *H. pylori*. The biopsy samples (3×3 cm) from gastric pylorus were minced, applied to CLO kits (Kimberly-Clark, Roswell, GA, USA), and incubated at 35°C for 24 hours to examine urease activity. The reaction (color change) was determined as negative for bright yellow, false (partially) positive for thick yellow, or positive for thick (dark) red [16,17, 33,34].

Among total of 60 patients (20-70 years old) who visited the Gastroenterology Department of the Catholic University Medical Center in Daegu, 42 patients (21 males, 21 females) were enrolled in this study (Table 1). Enrollment criteria included infection of *H. pylori* which were confirmed by gastric endoscopy and UBT. Exclusion criteria included patients with experience of *H. pylori* treatment, administration of NSAID or antibiotics within 4 weeks, heart failure, liver cirrhosis, severe cardiovascular, brain or renal diseases as well as pregnant or nursing women. The study protocol was approved by the Institutional review board (IRB) of the Catholic University Medical Center in Daegu (Approval No. CR-10-074-RES-01-R), and written informed consent was obtained from all patients.

We designed a randomized, double-blind, placebo-controlled clinical trial for eradication therapy of *H. pylori*. The patients in treatment group were given twice a day 1 FEMY-R7 capsule containing 75 mg fucoidan and 75 mg evening primrose extract (300 mg/day) before a meal for 8 weeks. The patients in placebo control group were given only the capsules containing 150 mg microcrystalline cellulose. During the treatment period, the patients were requested to keep their own dietary lifestyle.

The presence of *H. pylori* in the stomach was detected by <sup>13</sup>C-UBT [35,36]. Briefly, after 8-hour fasting, breath sample was collected in aluminized bag as the baseline value, followed by ingestion of 75 mg of <sup>13</sup>C-urea powder (Helikit™; Isotechnika Inc, Edmonton, Canada) in 70 mL drinking water. Thirty min later, breath samples were collected, and analyzed by an isotope mass spectrometer (Heliview; Medichems, Seoul, Korea) to measure the <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> ratio. A Delta over baseline-value (DOB) risen in the exhaled air by ≥4.0% was considered

Table 1. Basic information on the human subjects

Treatment (mg/day)	Number of patients	Age	Male/female
Placebo	21	45.6±2.8	11/10
FEMY-R7 (300)	21	47.2±3.0	11/10

positive for *H. pylori* infection according to the manufacturer instruction and previous reports [35-37].

The fasting serum samples were collected from the patients, and both serum pepsinogen I and pepsinogen II levels were determined by a latex-enhanced turbidimetric immunoassay (HBi Co., Anyang, Korea) using an automated Toshiba-200FR system (Toshiba Medical Systems Co., Tokyo, Japan) [38-40].

Data were expressed as the mean±SEM. Statistical analysis was performed using an analysis of variance (ANOVA) followed by the Dunnett's multiple-range test correction with the aid of SPSS for Windows v.10.0 (Chicago, IL, USA). A *P* value <0.05 was considered statistically significant.

Repeated intragastric inoculation (1×10<sup>9</sup> CFU/mouse, 3 times) of *H. pylori* to C57BL/6 mice revealed positive reaction (red color) in CLO test. The mice orally treated with 10 or 100 mg/kg FEMY-R7 twice a day for 2 weeks displayed positive reaction in 33.3% (2/6) and 16.7% (1/6) mice, respectively (Table 2). FEMY-R7 at 200 mg/kg/day near-fully eliminated the bacteria from the gastric wall, which was also confirmed in our previous study [33].

Based on the high effectiveness of FEMY-R7 in mice experimentally infected with *H. pylori*, we performed a clinical trial in naturally-infected patients. The initial conditions including age and gender were similar between placebo and treatment groups (Table 1). As analyzed by UBT, the BOD of patients treated with FEMY-R7 (300 mg/day) significantly decreased by 31.8% and 42% at 4 and 8 weeks, respectively (Figure 1), in comparison with no change in placebo group.

Notably, 8-week treatment with FEMY-R7 significantly decreased the serum pepsinogen I level, an atrophic gastritis marker (Table 3) [41,42]. Especially, FEMY-R7

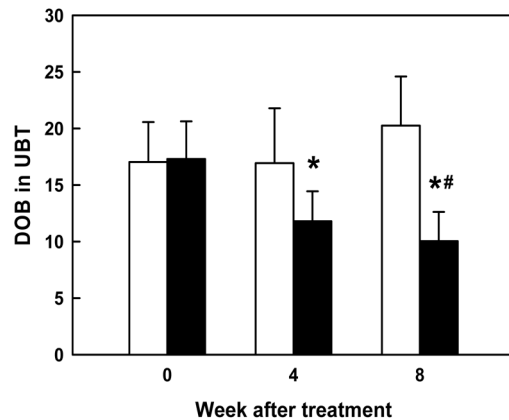


Figure 1. Effect of FEMY-R7 (300 mg/day) on the Delta over baseline-value (DOB, %) in urea breath test (UBT) on the expiratory breath of patients infected with *H. pylori*. White: placebo, black: FEMY-R7. \*Significantly different from before treatment (0 time). #Significantly different from placebo (8 week).

more markedly reduced the pepsinogen II level, a surrogate marker of inflammatory response to *H. pylori* infection [38-40], leading to the increase in the pepsinogen I/II ratio. By comparison, both the serum pepsinogen I and II levels were not affected following intake of placebo capsules. On the other hand, FEMY-R7 did not affect the hematology [white blood cells, red blood cells (RBC), RBC indices, and platelets] and blood biochemistry related to liver function, kidney function, and energy and lipid metabolism (data not shown).

*H. pylori* is known to secrete urease to survive in acidic environment and to invade gastric mucosa, causing gastritis, ulcers, and sometimes development of gastric malignancies. In a previous [33] and the present investigations, we demonstrated that FEMY-R7 has an anti-bacterial activity against *H. pylori*. In CLO test, treatment with FEMY-R7 for 2 weeks eliminated *H. pylori* from the stomach of mice in a dose-dependent manner, displaying a near-full efficacy at 200 mg/kg/day. It is of interest to note that the *H. pylori*-eradicating effect of 200 mg/kg/day FEMY-R7 was comparable to that of 60 mg/kg/day pantoprazole, a well-known proton pump inhibitor [33]. More importantly, the effectiveness of FEMY-R7 was also confirmed in humans; i.e., daily

Table 2. Effect of FEMY-R7 on the reactivity in CLO test on the gastric mucosa of mice infected with *H. pylori*

Treatment (mg/kg/day)	1	2	3	4	5	6	Positive ratio (%)
Vehicle control	●	●	●	●	●	●	6/6 (100)
+FEMY-R7 (20)	●	◐	○	○	○	○	2/6 (33.3)
+FEMY-R7 (200)	◐	○	○	○	○	○	1/6 (16.7)

○, negative; ◐, partially positive; ●, positive.

**Table 3.** Effect of FEMY-R7 on the serum pepsinogen levels of patients infected with *H. pylori*

Treatment (mg/day)	Time after treatment (week)	Pepsinogen I ( $\mu\text{g/mL}$ )	Pepsinogen II ( $\mu\text{g/mL}$ )	Pepsinogen I/II ratio
Placebo	0	66.11 $\pm$ 5.25	19.35 $\pm$ 1.76	3.56 $\pm$ 0.29
	8	69.81 $\pm$ 4.77	21.74 $\pm$ 2.10	3.52 $\pm$ 0.31
FEMY-R7 (300)	0	66.28 $\pm$ 3.73	19.56 $\pm$ 1.56	3.59 $\pm$ 0.19
	8	48.72 $\pm$ 3.52**	12.13 $\pm$ 1.27**	4.45 $\pm$ 0.25*

\*Significantly different from before treatment (0 time). \*\*Significantly different from placebo (8 week).\

intake of 300 mg FEMY-R7 for 8 weeks significantly lowered BOD in the expiratory breath.

In our previous study [33], it was found that FEMY-R7 eliminates *H. pylori* from the gastric walls by killing the bacteria and inhibiting their invasion, but not by preserving or strengthening the mucosal layer including mucus content. Actually, fucoidan and evening primrose extract inhibited the adhesion of *H. pylori* to gastric cells and their colonization there [29,30,32], which is a well-known action mechanism of proton-pump inhibitors that suppress *H. pylori* growth [43-45].

Notably, FEMY-R7 treatment decreased the blood levels of pepsinogens I and II. Since a part (approximately 1%) of the pepsinogens secreted from the stomach walls are released into blood, the levels of pepsinogens I and II and their ratio have been used as a surrogate marker of gastric secretory function [46-48]. Especially, the blood pepsinogen II level was proposed to be a potential marker of morphological change of the stomach during *H. pylori*-induced gastritis and tumor development [39]. Indeed, in previous studies [38,40], successful eradication therapy of *H. pylori* resulted in a decrease in the serum level of pepsinogen II, and ensuing an increase in the pepsinogen I/II ratio. In the present study, both the blood pepsinogens I and II decreased and the ratio of pepsinogen I/II increased following treatment with FEMY-R7, indicating that it improved the inflammatory response by eliminating *H. pylori* from the gastric mucosa.

Effective therapy of *H. pylori* has been provided with triple therapies (for example, omeprazole+clarithromycin+amoxicillin or omeprazole+clarithromycin+metronidazole) consisting of two antibiotics [2]. However, it has been well demonstrated that the widespread use of antibiotics led to a rapidly-increasing bacterial resistance [24,43]. Thus, there is a strong rationale for the development of novel anti-bacterial agents without resistance and adverse-effects. In the present study, it was found that FEMY-R7 not only eliminated *H. pylori* from the stomach walls of mice and humans, but also did not alter the hematological and blood biochemical parameters, related to tissue

injuries, of human patients. Taken together, it is suggested that FEMY-R7, a combinational regimen composed of fucoidan and evening primrose extract, could be a promising candidate overcoming tolerance of antibiotics for the treatment of recurrent *H. pylori* infection.

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