Role of Pediococcus Acidilactici J9 in Decreasing the Occurrence of Gastritis Caused by H.pylori Infection and Two-week Repeated-dose Oral Toxicity Study in Mice

Mijung Lee
Seoul National University Hospital https://orcid.org/0000-0002-7709-7766
Jin-Young Chung
Kangwon National University College of Veterinary Medicine
Ka Yeun Kim
Fordham University
Wooseok Im
Seoul National University Hospital
Manho Kim (✉ kimmanho@snu.ac.kr)
https://orcid.org/0000-0002-0277-6326

Research

Keywords: Pediococcus acidilactici J9, repeated two-week oral dose toxicity, mouse, Helicobacter pylori, AGS cell

Posted Date: May 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-25937/v1

Abstract

BACKGROUND Helicobacter pylori (*H. pylori*) is an important pathogen that causes chronic gastritis and peptic ulcer, and is related to the development of gastric carcinoma. Several chemicals, including antibiotics, have been used to eradicate *H.pylori*. However, more studies are yet requred to accomplish a sufficient therapy. *Pediococcus acidilactici* J9 were studied for inhibition of binding of *H.pylori* binding to human gastric cell lines. This study was performed in order to investigate the repeated-dose toxicity of *Pediococcus acidilactici* J9 in male and female mice.

RESULTS C57BL/6 male and female *Mus musculus* were divided into four groups (n = 10 in each group). *Pediococcus acidilactici* J9 was administered daily by oral injection of vehicle control at dosage levels to a low-dose group (500 mg/kg/day), middle-dose group (1000 mg/kg/day), and high-dose group (2000 mg/kg/day) for two weeks. After 14 days of exposure, the blood biochemistry and hematology were investigated, along with a histopathology exam. There were no bacterial-related deaths or abnormal clinical signs in either gender of mouse. The data was observed during the period in terms of body weight, food, intake, and water consumption. Also, no alterations in organ weights upon administration of *Pediococcus acidilactici* J9 alone were observed.

CONCLUSIONS These results suggest that the oral application of *Pediococcus acidilactici* J9, up to a dosage level of 2,000 mg/kg/day, causes no adverse effects in both male and female mice. *Pediococcus acidilactici* J9 inhibits the adhesion of *H.pylori* to AGS gastric cancer cells. When used as probiotics, *Pediococcus acidilactici* J9 may help decrease the occurrence of gastritis and reduce the risk of *H.pylori* infection with promising safety issues.

Background

Lactobacillus is a gram-positive microorganism that utilizes carbohydrates as the energy source and produces organic acids like lactic acid and acetic acid as final products. It is used industrially in various fermented products, like fermented vegetables and dairy products, which are broadly involved in the everyday life [1, 2]. Lactobacillus determines the flavor of fermented foods and the characteristics of fermented products, and it plays a critical role in the food preservation. This occurs by extending its shelf life via production of active antibiotic materials like organic acid and bacteriocin. Moreover, various function in the human body is also reported, such as the suppression of intestinal noxious bacteria, the decrease of blood cholesterol levels, anticancer effect, reinforcement of immune function, etc. Because of the Lactobacillus, as a probiotics, intakes living strain, it attracts attention as an antibacterial preparation that solves the residual tolerance problems, in addition to being recently utilized as a healthy functional food [3, 4].

Helicobacter pylori is a macroaerophilic gram-negative bacteria that causes chronic gastritis, peptic ulcer, and presumable gastric cancer. Accumulated evidence demonstrates that the eradication of these bacteria resolves *H.pylori*-associated disease [5]. Multicenter studies have shown that triple therapy via a proton pump inhibitor (PPI), clarithromycin, and either amoxicillin or metronidazole (all taken twice daily). This therapy is among the most effective approaches to *H. pylori* eradication [6]. However, 5–10% of *H. pylori* strains are reportedly resistant to clarithromycin [7]. In addition, there was a study noted a clarithromycin-resistant mutation in 63% of *H. pylori* strains from patients in whom treatment with a regimen including clarithromycin was unsuccessful [8]. The treatment of *H. pylori* infection with antibiotics does not eradicate the organism and is also often

accompanied by deleterious side effects [9]. Thus, although many experts believe that “untreatable” *H. pylori* is just ill-treated *H. pylori*, no clinical trial. To the best of our knowledge, *H. pylori* has not yielded a treatment that provides 100% eradication [10]. Recently, probiotic lactic acid bacteria (LAB) have been reported to control *H. pylori*.. Also, several studies have examined the efficacy of various probiotic preparations for *H. pylori* eradication with and without co-interventions [11]. Moreover, a number of clinical trials have been undertaken to test the hypothesis that probiotic bacteria inhibits *H. pylori* infection [12]. Probiotics inhibit enteric pathogens such as *Salmonella*, *Shigella*, and *Citrobacter rodentium* in both *in vitro* and *in vivo* [13, 14], and potential clinical benefits in preventing or resolving gastrointestinal diseases have been demonstrated [15, 16].

These microorganisms provide gut protection through several mechanisms, including decreasing luminal pH by producing lactic acid [17, 18] and competing with gut pathogens for host surface receptors [19]. Nonetheless, it has been shown that probiotics may inhibit *H. pylori* growth, independent of pH and lactic acid levels [20]. *In vitro* assays were carried out to determine whether the combination of *Pediococcus acidilactici* J9, and its adhesion to gastric cells thus impacting gastric acidity, inhibit the growth of *H. pylori*. The current therapeutic regimen for *H. pylori* aims to eliminate bacterial growth with antibiotics and this reduces the total acidity of gastric acid.

In this study, repeated toxicity tests are performed as the stability test. using mice of C57BL/6 type under the “standard of toxicity test for medicine and medical supplies (Korea food and drug administration notification No. 1999-61)”. We also demonstrated in in vitro models that *Pediococcus acidilactici* J9 in combination have beneficial effects similar to those of antibiotic therapy on *H. pylori*-infected gastric epithelium.

Results

Death rate and normal symptoms

Pediococcus acidilactici J9 was administrated by oral injection for 2 weeks and the Table 1, 2 show the death rate and the normal symptoms of males and females observed for 2 weeks. During the experiment, experimental mice were observed at regular times, and no death was observed in the male and female administration group (Table 1). Also, during all of the experiment, in every administration group – low dose (500 mg/kg/day), medium dose (1,000 mg/kg/day), and high dose (2,000 mg/kg /day) - including the control group, no specific adverse symptoms are observed (Table 2). In this study, a dose of 2,000 mg/kg, which is a maximum dose of oral administration toxicity test, did not generate abnormal symptoms. It thus seems that the minimal lethal dose of this experimental materials exceeds 2,000 mg/kg/day in both male and female.

Table 1
Mortality of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Dose (mg)	No. of animal	Days after treatment				Final mortality
			0	7	14	End	
Male	0	10	0/10	0/10	0/10	T.S	0/10
	500	10	0/10	0/10	0/10	T.S	0/10
	1,000	10	0/10	0/10	0/10	T.S	0/10
	2,000	10	0/10	0/10	0/10	T.S	0/10
Female	0	10	0/10	0/10	0/10	T.S	0/10
	500	10	0/10	0/10	0/10	T.S	0/10
	1,000	10	0/10	0/10	0/10	T.S	0/10
	2,000	10	0/10	0/10	0/10	T.S	0/10
Values are expressed as the numbers of dead animals/total numbers of animals							
T.S: terminal sacrifice.							

Table 2
Clinical signs of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Dose (mg)	Clinical signs	Days after treatment		
			0	7	14
Male	0	NAD	0/10	0/10	0/10
	500	NAD	0/10	0/10	0/10
	1,000	NAD	0/10	0/10	0/10
	2,000	NAD	0/10	0/10	0/10
Female	0	NAD	0/10	0/10	0/10
	500	NAD	0/10	0/10	0/10
	1,000	NAD	0/10	0/10	0/10
	2,000	NAD	0/10	0/10	0/10
NAD: no abnormalities detected.					
Values are expressed as number of animals with the sign/number of animals examined.					

Changes In Body Weight

Pediococcus acidilactici J9 was orally administrated for 2 weeks with varying concentrations and the changes in body weight are shown in Table 3. Changes in body weight during the whole period of experiment were negligible for the control group, low dose group (500 mg/kg/day), medium dose group (1,000 mg/kg/day), and high dose group (2,000 mg/kg/day). Additionally, from the date of administration of experimental materials to the end of the experiment, there was a normal weekly increase in body weight in the control group and the administration group (Fig. 1A).

Table 3
Body weight changes of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Dose (mg)	Body weight (g)		
		Days after treatment		
		0	7	14
Male	0	19.07 ± 0.80^{NS}	19.64 ± 0.95 ^{NS}	20.62 ± 1.13 ^{NS}
	500	18.79 ± 1.32	19.40 ± 1.53	20.22 ± 1.39
	1,000	19.21 ± 0.70	19.90 ± 1.04	20.59 ± 1.11
	2,000	19.08 ± 0.92	19.89 ± 1.50	20.83 ± 1.79
Female	0	16.60 ± 0.96^{NS}	16.67 ± 0.77^{NS}	17.79 ± 0.77^{NS}
	500	16.64 ± 0.64	16.94 ± 0.40	17.49 ± 0.47
	1,000	16.41 ± 0.64	16.78 ± 0.71	17.37 ± 0.79
	2,000	16.30 ± 0.79	16.16 ± 0.96	16.92 ± 0.85
Values are expressed as mean ± SE (n = 10).				
NS: not significantly different among groups.				

Intake Of Nutrition And Water

There was no significant change in the control group and the experimental material administration group in the amount of intake of feed and water during the experiment period (Tables 4 and 5). Therefore, it seems that the administration of experimental materials does not affect significantly the amount of intake of feed and water (Fig. 1B and C).

Table 4
Daily food consumption of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Dose (mg)	Food consumption (g/day/mouse)	
		Days after treatment	
		7	14
Male	0	74 NS	87NS
	500	92	91
	1,000	71	95
	2,000	113	93
Female	0	103 NS	83NS
	500	85	81
	1,000	108	79
	2,000	65	70
Values are expressed as mean (n = 2).			
NS: not significantly different among groups.			

Table 5
Daily water consumption of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Dose (mg)	Water consumption (mL/day/mouse)	
		Days after treatment	
		7	14
Male	0	139 ± NS	120 ± NS
	500	141±	118±
	1,000	137±	111±
	2,000	154±	137±
Female	0	127 ± NS	124 ± NS
	500	148±	129±
	1,000	142±	152±
	2,000	132±	112±
Values are expressed as mean (n = 2).			
NS: not significantly different among groups.			

Autopsy Results

As the result of the observation of main organs with naked eyes after the autopsy of experimental mice, there was no significant change in organs and specific autopsy opinion dependent on the dose of administration (Table 6). However in both control group and administration group, blackish red discoloration at the spleen terminal, shrinkage of the right testicle, and thinning of the right atrium were observed. (CTR-F-001: discoloration of spleen; CTR-F-005: discoloration of spleen; 500 mg-F-001: discoloration of spleen; CTR-M-004: discoloration of spleen; CTR-M-005: thinning of right atrium; 500 mg-M-004: discoloration of spleen; 1000 mg-M-004: shrinkage of right testicle)

Table 6
Gross findings of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex		**Male**				**Female**			
Dose (mg)		0	500	1,000	2,000	0	500	1,000	2,000
Brain	NGF	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
Lung	NGF	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
Liver	NGF	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
Heart	NGF	9(90)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
Kidney(L)	NGF	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
Kidney(R)	NGF	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
Testis(L)	NGF	10(100)	10(100)	10(100)	10(100)				
Testis(R)	NGF	10(100)	10(100)	9(90)	10(100)				
Ovary(L)	NGF					10(100)	10(100)	10(100)	10(100)
Ovary(R)	NGF					10(100)	10(100)	10(100)	10(100)
Spleen	NGF	9(90)	9(90)	10(100)	10(100)	7(70)	9(90)	10(100)	10(100)
Thymus	NGF	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)	10(100)
NGF: no gross finding									
Values are expressed as animal numbers (the percentage of animal numbers)									

The Weight Of Organs

The weight of organs were measured after repeated administration of *Pediococcus acidilactici* J9 which varied to low dose (500 mg/kg/day), medium dose (1,000 mg/kg/day), and high dose (2,000 mg/kg/day) for 2 weeks (Table 7). No changes were observed in the weight of brain, lung, testis, ovary, kidney, heart, spleen, and liver with respect to the administration of experimental materials and no abnormal changes were dependent on dose of administrations. Generally, when the toxic materials were ingested, liver takes the largest effect since the detoxification starts at the liver. However, there were no significant changes in each group on the observed

weight of the liver. From the results above, the administration of *Pediococcus acidilactici* J9 does not affect the weight of organs.

Table 7
Organ weights of male and female (mice) treated orally with Pediococcus acidilactici J9 for 14 days

Sex	Organs	Dose (mg)			
		0	500	1,000	2,000
Male	Brain	0.445 ± 0.014	0.440 ± 0.014	0.444 ± 0.015	0.437 ± 0.016
	Lung	0.124 ± 0.013	0.126 ± 0.006	0.125 ± 0.010	0.134 ± 0.023
	Liver	0.984 ± 0.208	0.920 ± 0.065	0.929 ± 0.183	0.904 ± 0.176
	Heart	0.104 ± 0.011	0.101 ± 0.007	0.100 ± 0.008	0.105 ± 0.010
	Kidney(L)	0.129 ± 0.025	0.128 ± 0.025	0.127 ± 0.13	0.126 ± 0.018
	Kidney(R)	0.133 ± 0.025	0.136 ± 0.011	0.134 ± 0.017	0.140 ± 0.029
	Testis(L)	0.071 ± 0.020	0.067 ± 0.000	0.067 ± 0.008	0.071 ± 0.006
	Testis(R)	0.074 ± 0.024	0.070 ± 0.000	0.063 ± 0.018	0.074 ± 0.007
	Spleen	0.057 ± 0.009	0.052 ± 0.007	0.050 ± 0.006	0.054 ± 0.014
	Thymus	0.042 ± 0.010	0.046 ± 0.007	0.044 ± 0.007	0.045 ± 0.008
Female	Brain	0.443 ± 0.014	0.434 ± 0.019	0.437 ± 0.013	0.431 ± 0.018
	Lung	0.120 ± 0.006	0.120 ± 0.008	0.134 ± 0.014	0.121 ± 0.017
	Liver	0.775 ± 0.065	0.788 ± 0.199	0.755 ± 0.092	0.727 ± 0.103
	Heart	0.091 ± 0.007	0.089 ± 0.005	0.105 ± 0.007	0.084 ± 0.007
	Kidney(L)	0.114 ± 0.025	0.102 ± 0.011	0.126 ± 0.007	0.102 ± 0.010
	Kidney(R)	0.110 ± 0.011	0.110 ± 0.013	0.140 ± 0.012	0.105 ± 0.012
	Ovary (L)	0.001 ± 0.000	0.002 ± 0.000	0.071 ± 0.000	0.001 ± 0.000
	Ovary(R)	0.002 ± 0.000	0.002 ± 0.000	0.074 ± 0.000	0.002 ± 0.000
	Spleen	0.058 ± 0.007	0.052 ± 0.010	0.053 ± 0.010	0.051 ± 0.007
	Thymus	0.077 ± 0.007	0.071 ± 0.010	0.045 ± 0.011	0.076 ± 0.014
Values are expressed as mean ± SE (n = 10).					
NS: not significantly different among groups.					

Hematological Tests

As the result of the measurement of percentages of Red blood cells, RBC, hematocrit, HCT, hemoglobin, Hb, mean corpuscular volume, MCV, mean corpuscular hemoglobin, MCH, mean corpuscular hemoglobin concentration, MCHC, white blood cells, WBC, Hemoglobin, HGB, Cellular Hb Concentration Mean, CHCM, Red Cell Distribution Width, RDW, Hb Distribution Width, HDW, Cellular Hb content, CH, Cellular Hb Distribution Width, CHDW, Platelet, PLT, Platelet Distribution Width, PDW, Plateletcrit, PCT, Neutrophil, NEUT, Neutrophil, NEUT%, Lymphocyte, LYMPH, Lymphocyte %, LYMPH%, Monocyte, MONO, Monocyte %, MONO%, Eosinophil, EOS, Eosinophil %, EOS%, Basophil, BASO, Basophil %, BASO%, Large Unstained Cells, LUC, Large Unstained Cells, LUC%, Reticulocyte Count, Retic#, Reticulocyte %, Retic%, Mean Corpuscular Volume of Retics, MCVr, Mean Corpuscular Volume of Retics %, MCVr%, Red Cell Distribution Width of Retics, RDWr*, Hb Distribution Width of Retics, HDWr*, Cellular Hb of Retics, CHr, and Cellular Hb Distribution Width of Retics, CHDWr* after the autopsy using blood auto-analyzer (System SE-9000, TOAMedical Electronics Co., Ltd., Kobe, Japan), no significant changes were observed in control and administration groups ($p \leq 0.05$) (Table 8). As a result of hematological examination, both the control group and the administration group were included in normal range and no dependence on dose was observed. This result is similar to the range previously reported in hematological fundamental database of which there is a repeated toxicity test for 2 weeks using mice.

Table 8
Hematology of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Parameters		Dose (mg)			
			0	500	1,000	2,000
Male	CBC	WBC (x10^3/μL)	2.672 ± 0.65	2.066 ± 0.63	2.016 ± 1.01	2.474 ± 0.96
		RBC (x10^6/μL)	9.88 ± 0.33	9.518 ± 0.26	10.00 ± 0.14	9.876 ± 0.32
		HGB (g/dL)	15.14 ± 0.64	14.72 ± 0.36	15.36 ± 0.28	15.26 ± 0.53
		HCT (%)	51.62 ± 2.24	49.42 ± 1.43	51.38 ± 1.17	50.90 ± 2.09
		MCV (fL)	52.26 ± 0.69	51.9 ± 0.42	51.36 ± 1.09	51.54 ± 0.72
		MCH (pg)	15.32 ± 0.22	15.46 ± 0.21	15.34 ± 0.22	15.46 ± 0.23
		MCHC (g/dL)	29.30 ± 0.23	29.78 ± 0.29	29.88 ± 0.43	29.96 ± 0.40
		CHCM (g/dL)	28.16 ± 0.35	28.16 ± 0.11	28.64 ± 0.44	28.68 ± 0.45
		RDW (%)	13.36 ± 0.38	13.88 ± 0.40	13.30 ± 0.50	13.26 ± 0.94
		HDW (g/dL)	1.45 ± 0.02	1.45 ± 0.06	1.45 ± 0.04	1.45 ± 0.03
		CH (pg)	14.68 ± 0.19	14.58 ± 0.08	14.66 ± 0.17	14.74 ± 0.09
		CHDW (%)	2.00 ± 0.05	2.07 ± 0.08	2.00 ± 0.05	2.01 ± 0.11
		PLT (x10^3/μL)	1263.20 ± 73.52	1244.80 ± 57.87	1238.60 ± 61.75	1202.00 ± 60.76
		MPV (fL)	7.44 ± 0.17	7.88 ± 0.05	7.52 ± 0.08	7.48 ± 0.26
		PDW (%)	60.38 ± 1.85	57.30 ± 1.62	55.80 ± 1.62	55.96 ± 2.34
		PCT (%)	0.94 ± 0.06	0.98 ± 0.05	0.93 ± 0.05	0.90 ± 0.06
	DIFF	NEUT (x10^3/μL)	0.34 ± 0.13	0.18 ± 0.05	0.19 ± 0.09	0.21 ± 0.05
		NEUT (%)	12.5 ± 3.19	9.26 ± 2.15	9.56 ± 1.06	8.94 ± 1.50
		LYMPH (x10^3/μL)	2.25 ± 0.52	1.83 ± 0.56	1.78 ± 0.89	2.22 ± 0.89
		LYMPH (%)	84.46 ± 3.46	88.48 ± 2.27	88.52 ± 1.21	89.46 ± 1.17
		MONO (x10^3/μL)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
		MONO (%)	0.54 ± 0.34	0.32 ± 0.28	0.28 ± 0.18	0.32 ± 0.16
		EOS (x10^3/μL)	0.05 ± 0.02	0.03 ± 0.04	0.02 ± 0.01	0.01 ± 0.01
		EOS (%)	1.64 ± 0.50	1.30 ± 1.31	0.76 ± 0.30	0.48 ± 0.21

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.

		BASO (x10^3/μL)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
		BASO (%)	0.28 ± 0.13	0.40 ± 0.20	0.34 ± 0.24	0.26 ± 0.19
		LUC (x10^3/μL)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
		LUC (%)	0.62 ± 0.16	0.24 ± 0.27	0.48 ± 0.33	0.56 ± 0.23
	RETI	Reticulocyte (x10^9/μL)	301.60 ± 47.96	258.32 ± 30.72	302.89 ± 10.26	298.02 ± 63.69
		Reticulocyte (%)	3.15 ± 0.51	2.71 ± 0.25	3.03 ± 0.12	3.00 ± 0.56
		MCVr (fL)	58.00 ± 0.51	57.46 ± 0.48	57.60 ± 0.65	57.86 ± 1.13
		CHCMr (g/dL)	26.24 ± 0.17	25.92 ± 0.13	26.30 ± 0.19	26.18 ± 0.31
		RDWr (%)	12.06 ± 0.65	12.36 ± 0.66	11.96 ± 0.64	12.20 ± 0.85
		HDWr (%)	2.34 ± 0.12	2.40 ± 0.10	2.43 ± 0.07	2.52 ± 0.10
		CHr (pg)	15.20 ± 0.25	14.86 ± 0.22	15.12 ± 0.11	15.12 ± 0.16
		CHDWr (%)	1.98 ± 0.08	1.97 ± 0.07	1.92 ± 0.05	1.97 ± 0.06
Female	CBC	WBC (x10^3/μL)	2.95 ± 0.60	2.53 ± 1.08	2.75 ± 0.70	3.32 ± 1.34
		RBC (x10^6/μL)	9.51 ± 0.13	10.19 ± 0.15	10.07 ± 0.22	9.97 ± 0.31
		HGB (g/dL)	14.72 ± 0.21	15.80 ± 0.47	15.60 ± 0.26	15.74 ± 0.31
		HCT (%)	49.12 ± 0.73	52.20 ± 1.70	51.48 ± 1.52	50.44 ± 1.77
		MCV (fL)	51.64 ± 0.43	51.16 ± 1.04	51.12 ± 0.79	50.60 ± 0.62
		MCH (pg)	15.50 ± 0.23	15.48 ± 0.33	15.50 ± 0.33	15.78 ± 0.42
		MCHC (g/dL)	29.98 ± 0.36	30.28 ± 0.35	30.32 ± 0.61	31.20 ± 0.91
		CHCM (g/dL)	28.74 ± 0.34	28.94 ± 0.40	28.96 ± 0.43	29.5 ± 0.35
		RDW (%)	13.68 ± 0.46	13.90 ± 0.46	13.00 ± 0.52	13.02 ± 0.46
		HDW (g/dL)	1.50 ± 0.05	1.54 ± 0.03	1.52 ± 0.04	1.59 ± 0.02
		CH (pg)	14.76 ± 0.13	14.74 ± 0.21	14.76 ± 0.15	14.86 ± 0.06
		CHDW (%)	2.07 ± 0.06	2.11 ± 0.05	2.00 ± 0.08	2.03 ± 0.06
		PLT (x10^3/μL)	955.60 ± 64.27	1102.40 ± 63.27	1129.80 ± 94.82	1035.00 ± 107.62
		MPV (fL)	7.56 ± 0.35	7.70 ± 0.16	7.82 ± 0.11	7.38 ± 0.47
		PDW (%)	57.78 ± 3.04	57.10 ± 2.82	54.94 ± 1.31	57.68 ± 3.19

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.

	PCT (%)	0.73 ± 0.07	0.85 ± 0.06	0.89 ± 0.08	0.77 ± 0.11
DIFF	NEUT (x10^3/μL)	0.28 ± 0.07	0.29 ± 0.06	0.25 ± 0.08	0.23 ± 0.12
	NEUT (%)	9.48 ± 0.42	12.38 ± 2.50	9.70 ± 3.01	6.94 ± 1.34
	LYMPH (x10^3/μL)	2.61 ± 0.53	2.20 ± 1.01	2.45 ± 0.72	3.03 ± 1.19
	LYMPH (%)	88.50 ± 0.39	86.24 ± 2.40	88.74 ± 3.30	91.42 ± 1.61
	MONO (x10^3/μL)	0.01 ± 0.00	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
	MONO (%)	0.46 ± 0.15	0.20 ± 0.12	0.22 ± 0.13	0.22 ± 0.08
	EOS (x10^3/μL)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
	EOS (%)	0.80 ± 0.25	0.34 ± 0.38	0.34 ± 0.26	0.42 ± 0.29
	BASO (x10^3/μL)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
	BASO (%)	0.32 ± 0.22	0.30 ± 0.12	0.38 ± 0.19	0.30 ± 0.19
	LUC (x10^3/μL)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
	LUC (%)	0.48 ± 0.13	0.54 ± 0.18	0.64 ± 0.51	0.66 ± 0.38
RETI	Reticulocyte (x10^9/μL)	309.34 ± 52.93	304.60 ± 43.32	277.50 ± 38.28	351.10 ± 49.29
	Reticulocyte (%)	3.25 ± 0.53	2.99 ± 0.42	2.76 ± 0.41	3.53 ± 0.60
	MCVr (fL)	57.90 ± 0.50	57.86 ± 1.25	57.36 ± 0.70	58.12 ± 0.75
	CHCMr (g/dL)	26.46 ± 0.18	26.42 ± 0.33	26.44 ± 0.11	26.76 ± 0.23
	RDWr (%)	13.08 ± 0.93	12.14 ± 1.01	13.40 ± 0.70	13.22 ± 1.00
	HDWr (%)	2.58 ± 0.11	2.63 ± 0.11	2.75 ± 0.12	2.81 ± 0.12
	CHr (pg)	15.30 ± 0.23	15.24 ± 0.42	15.12 ± 0.22	15.52 ± 0.13
	CHDWr (%)	2.03 ± 0.04	2.01 ± 0.08	2.07 ± 0.07	2.13 ± 0.10

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.

Blood biochemical analysis.

As the result of the measurement of Glucose; GLU, Blood Urea Nitrogen; BUN, Creatinine; CREA, Total cholesterol; T-CHOL, Albumin; ALB, *Total Bilirubin; T-BIL*, Alkaline Phosphatase; ALP, Aspartate Aminotransferase; AST(GOT), Alanine Aminotransferase; ALT(GPT), Triglyceride; TG, Total protein; TP using auto-analyzer (Hitachi-747, Hitachi Medical Co., Tokyo, Japan) from blood serum, which is the indicator of blood biochemistry, no significant changes dependent on the administration of experimental materials were observed in the whole administration

groups with respect to the control group. Both the control group and *Pediococcus acidilactici* J9 administration group showed normal parameters ($p \leq 0.05$) (Table 9).

Table 9
Levels of serum biochemical indices of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

Sex	Parameters	Dose (mg)			
		0	500	1,000	2,000
Male	Glu	256.40 ± 11.91	243.00 ± 42.57	234.20 ± 25.82	230.80 ± 31.32
	BUN	18.68 ± 3.87	21.54 ± 5.78	20.50 ± 6.22	18.24 ± 2.60
	Crea	0.28 ± 0.04	0.29 ± 0.02	0.27 ± 0.02	0.27 ± 0.03
	T-Chol	79.00 ± 4.36	85.20 ± 4.92	82.40 ± 6.80	81.80 ± 4.66
	TP	4.74 ± 0.05	4.82 ± 0.24	4.76 ± 0.11	4.68 ± 0.16
	ALB	1.70 ± 0.07	1.72 ± 0.08	1.68 ± 0.04	1.68 ± 0.13
	T-BIL	0.06 ± 0.05	0.04 ± 0.05	0.08 ± 0.04	0.08 ± 0.04
	ALP	133.00 ± 19.51	137.80 ± 9.65	126.20 ± 19.82	137.40 ± 15.96
	AST(GOT)	56.20 ± 15.07	65.20 ± 13.18	48.00 ± 7.35	57.40 ± 13.28
	ALT(GPT)	28.80 ± 4.15	28.00 ± 4.18	22.20 ± 1.30	25.20 ± 4.49
	TG	66.40 ± 26.88	64.40 ± 31.45	49.80 ± 23.85	39.40 ± 17.94
	A/G	0.56 ± 0.05	0.56 ± 0.05	0.52 ± 0.04	0.56 ± 0.05
	B/C	66.61 ± 12.59	74.13 ± 18.80	76.83 ± 22.46	69.05 ± 11.10
Female	Glu	214.40 ± 37.40	229.60 ± 47.45	228.40 ± 40.32	231.00 ± 66.87
	BUN	25.16 ± 5.42	24.70 ± 5.07	23.06 ± 3.26	22.58 ± 4.50
	Crea	0.27 ± 0.05	0.25 ± 0.05	0.28 ± 0.03	0.26 ± 0.03
	T-Chol	76.80 ± 8.11	70.20 ± 5.97	78.80 ± 13.44	80.00 ± 9.85
	TP	4.82 ± 0.08	4.72 ± 0.16	4.78 ± 0.20	4.70 ± 0.23
	ALB	1.74 ± 0.05	1.74 ± 0.05	1.74 ± 0.05	1.72 ± 0.04
	T-BIL	0.00 ± 0.00	0.02 ± 0.04	0.02 ± 0.04	0.02 ± 0.04
	ALP	168.20 ± 8.93	173.40 ± 10.31	154.20 ± 13.88	153.60 ± 26.37
	AST(GOT)	68.20 ± 17.02	67.60 ± 14.57	66.80 ± 8.20	78.40 ± 18.01
	ALT(GPT)	21.00 ± 12.88	22.80 ± 3.11	24.60 ± 3.51	23.00 ± 3.39
	TG	0.56 ± 0.05	43.00 ± 9.70	27.00 ± 3.00	34.20 ± 15.45
	A/G	0.56 ± 0.05	0.58 ± 0.04	0.56 ± 0.05	0.58 ± 0.04

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.

B/C	94.97 ± 12.02	103.82 ± 32.56	82.32 ± 13.29	87.08 ± 24.74
Values are expressed as mean ± SE (n = 5).				
NS: not significantly different among groups.				

Histopathology Observations

For the histopathology test of *Pediococcus acidilactici* J9-administrated mice, liver and kidney were stained by hematoxylin and eosin. As the result of the histopathology test, no lesions were observed in the liver, like infection, necrosis, iron pigmentation, and bilirubin pigmentation. The structure of liver cells were also normal (Fig. 2A). There was no lesions in the kidney, like infection and necrosis, and no changes were observed in kidney cells (Fig. 2B). Therefore, there is no significant changes in liver and kidney, and no extraordinary pathologic abnormality dependent on dose of experimental materials were observed in both the control group and administration group as the result of the histopathology test. This opinion seems to correspond with the long-term change of weight as well as the blood biochemical change.

Inhibition of adhesion and growth of H.pylori in gastric epithelial cells in the presence of Pediococcus acidilactici J9

The adhesion and growth of *H. pylori* were inhibited by a 24 h treatment of *H. pylori* and *Pediococcus acidilactici* J9 at a 200 ug / ml concentration on AGS cells, which are gastric cancer cells. Compared to the control group (AGS cell and H.pylori), the number of H. pylori analyzed by FACS significantly ($p < 0.01$) decreased after incubation of AGS cell with Pediococcus acidilactici J9 for 24 hours. Control biological triplicate groups are also analyzed for statistical options. (Fig. 3 and Fig. S1 in Additional file 1).

Discussion

Pediococcus acidilactici J9 was obtained from a bean paste soup prepared with ground fermented soybeans and beneficially affects to human body by improving its intestinal microbial balances. *Pediococcus acidilactici* J9 has been emerged as a potential probiotic. *Pediococcus acidilactici* J9 exerts as an antagonism against other enteric pathogens, primarily through the production of lactic acid and secretion of pediocin. Thermo-stable pediocin is an antimicrobial peptide is known to have a strong activity against food bacteria and pathogenic enteric bacteria [21]. For these reasons, Probiotics including *Pediococcus acidilactici* J9 were used for commercial healthcare products like beverages and foods. Pediocin secreted by *Pediococcus acidilactici* J9 has a potential to inhibit other pathogenic bacteria.

H.pylori is known to be an important causative agent of peptic ulcer, gastritis, gastric cancer, or mucosa associated lymphoid tissue lymphoma [22]. Various antibiotics have been used for *H.pylori* eradication [23]. These antimicrobial agents have been pointed out for various problems such as adverse effects, risk of re-infection due to increased pH, appearance of resistant bacteria, and high cost [24]. Recently, *H.pylori* inhibitory activity of natural products has been reported as a new treatment method of *H.pylori*. There is growing interest in probiotic lactic acid bacteria, which can play a role in the treatment of *H.pylori* by directly acting on *H.pylori*, with minimal clinical side effects of antibiotics [25].

This study investigated the toxicity and anti-*H.pylori* effect of *Pediococcus acidilactici* J9. Daily administration of *Pediococcus acidilactici* J9 in mice for two week showed no abnormal clinical signs in body weight, hematology, food intake and water consumption. In all test groups, no general symptoms and deaths from the test substance were observed. During the entire test period, body weight continuously increased but no significant change was observed with the control group. In addition, there were no significant differences in the gross observation, long-term weight change, hematology, blood biochemical and histopathologic examination of the organ in all the test substance administration groups, and all of them were within the normal range. As a result of repeated toxicity test for 2 weeks, *Pediococcus acidilactici* J9 was judged to be a safe and low-toxic substance. *Pediococcus acidilactici* J9, inhibits the adhesion of *H.pylori* to AGS gastric cancer cells. Probiotics refers to living microorganisms that are beneficial to the human body when consumed in moderate quantities [26, 27]. Most probiotics known to date are lactic acid bacteria [28, 29]. Probiotic bacteria such as lactic acid bacteria and beneficial bacteria survive in the stomach acid and bile acid in the body, reach the small intestine, multiply in the intestines and settle [30, 31]. It has a beneficial effect on health in the colon, and these probiotics should be non-toxic and non-pathogenic [32, 33]. Ingestion of probiotics not only helps maintain health, it also helps to improve various diseases such as infants, irritable bowel syndrome, and inflammatory bowel disease [34].

Based on our in vivo and in vitro results, when used as probiotics, *Pediococcus acidilactici* J9 may help decrease the occurrence of gastritis and reduce the risk of *H.pylori* infection with promising safety issues, without side effects.

Conclusions

In conclusion, we reported the toxicity and anti-*H.pylori* effect of *Pediococcus acidilactici* J9. Daily administration of *Pediococcus acidilactici* J9 in mice for two week showed no abnormal clinical signs in body weight, hematology, food intake and water consumption. Also, *Pediococcus acidilactici* J9, inhibited the adhesion of *H.pylori* to AGS gastric cancer cells. Based on our *in vivo* and *in vitro* results, when used as probiotics, *Pediococcus acidilactici* J9 may help decreasing the occurrence of gastritis and reducing the risk of *H.pylori* infection with promising safety issues, without side effects.

Methods

Model organisms and conditions

C57BL/6 mice of 4 weeks of age without certain pathogens are purchased at an amount of 20 males and females each. Normal and healthy mice without any weight loss are used in experiment by clinical observation during 7 days of education. Feeds are the following; solid feeds for laboratory animal are freely offered, and drinking water. The filtration-purified water is also freely offered to mice.

Configuration Of Test Group And Set Of Dosage Setting

Dosage was set by MFDs standards. Maximum dosage is set to 2,000 mg/kg/day for both male and female, with the geometric ratio of 1/2, low dose group, medium dose group, and high dose group are set at 500, 1000,

and 2000 mg per body weight(kg) respectively. The number of mice in each group are set to 5 males and females each. Dosage is set to not exceed 0.2 ml per 10 g and calculated according to the body weight measured just before administration. Test materials are well-mixed to sterile distilled water before administration, and they are directly injected into the stomach by sonde for oral administration for once a day during 2 weeks. Sterile distilled water for injection is used as reference material.

Normal Symptoms And Observation Of Lethality In Mice

Observation is conducted for 6 hours after oral administration and starting from the next day, to observe change of general condition, as well as expression of addiction. This was held in presence of dead mice and symptoms that can be expressed by the test materials are observed carefully. In the case of abnormality, the type and the extent of symptoms are recorded individually. All mice were checked for death or critical condition.

Measurement Of Weight, Feed And Water Intake

For every animal, change of weight is measured just before the administration of test materials once a week at a certain time during 2 weeks. Intake of feeds and water is measured and calculated weekly.

Autopsy And Naked Eye Examination

After administration going up to 14 days, the body weight of the surviving mice is measured, before anesthesia with CO2 and autopsy. External findings such as abnormality of subcutaneous, internal organs and brain were observed with the naked eye. The brain, kidney, liver, lung, reproductive organ, heart, spleen, and thymus are extracted and weighed.

Blood Biochemical

The hematologic analysis of the serum is performed the same day of the autopsy, which is collected from a 3,000 rpm, 20 minutes long, centrifugation of the blood and conducted by auto-analyzer (Hitachi-747, Hitachi Medical Co., Tokyo, Japan). Glucose; GLU, Blood Urea Nitrogen; BUN, Creatinine; CREA, Total cholesterol; T-CHOL, Albumin; ALB, *Total Bilirubin; T-BIL*, Alkaline Phosphatase; ALP, Aspartate Aminotransferase; AST(GOT), Alanine Aminotransferase; ALT(GPT), Triglyceride; TG, and Total protein; TP are measured.

Hematology

Mice fasted for 8 hours before the autopsy are slightly anesthetized with CO_2. Part of the blood from the exsanguination is EDTA-treated and stored in tubes and then analyzed by blood auto-analyzer (System SE-9000, TOAMedical Electronics Co., Ltd., Kobe, Japan). Red blood cells, RBC, hematocrit, HCT, hemoglobin, Hb, mean corpuscular volume, MCV, mean corpuscular hemoglobin, MCH, mean corpuscular hemoglobin concentration, MCHC, white blood cells, WBC, Hemoglobin, HGB, Cellular Hb Concentration Mean, CHCM, Red Cell Distribution Width, RDW, Hb Distribution Width, HDW, Cellular Hb content, CH, Cellular Hb Distribution Width, CHDW, Platelet,

PLT, Platelet Distribution Width, PDW, Plateletcrit, PCT, Neutrophil, NEUT, Neutrophil, NEUT%, Lymphocyte, LYMPH, Lymphocyte %, LYMPH%, Monocyte, MONO, Monocyte %, MONO%, Eosinophil, EOS, Eosinophil %, EOS%, Basophil, BASO, Basophil %, BASO%, Large Unstained Cells, LUC, Large Unstained Cells, LUC%, Reticulocyte Count, Retic#, Reticulocyte %, Retic%, Mean Corpuscular Volume of Retics, MCVr, Mean Corpuscular Volume of Retics %, MCVr%, Red Cell Distribution Width of Retics, RDWr*, Hb Distribution Width of Retics, HDWr*, Cellular Hb of Retics, CHr, and Cellular Hb Distribution Width of Retics, CHDWr* are measured.

Histopathology

Liver and kidney were extracted and fixed with a 10% neutral buffered formalin solution the day of final autopsy, after the observation of gross lesions on every animal which were administered with test materials. Then paraffin embedding wass conducted and hematoxylin & eosin dye performed with the sections of 3 ~ 4 um sections.

Helicobacter Pylori Preparation

Helicobacter pylori (ATCC 43504) used in this study were obtained and inoculated onto chocolate media, incubated for 5 ~ 7 days at 37°C in a 10% CO_2 incubator under aerobic conditions and then used for the examination. When the chocolate media is filled over 90%, *Helicobacter pylori* is swabbed with sterilized swabs and suspended in 20 ml of RPMI-1640 media to form the *Helicobacter pylori* suspension.

Cell Culture

The human gastric adenocarcinoma cell lines AGS (KCLB 21739; Korea) cells were seeded at a density of 1 × 10^5 cells in 2 ml of RPMI-1640 (RPMI-1640; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 1% penicillin-streptomycin (Invitrogen, USA) into 6 well culture plates (SPL) and cultured for 2 ~ 3 days at 37℃ in a 5% CO_2 incubator.

Adhesion Assay

When the AGS cell reach a density of 80% of the seeding plate, we eliminate the media from the plate and wash with phosphate buffered saline (PBS : Welgene, Daegu, Korea) 3 times. Experimental groups are as follows. For negative control, only AGS is seeded. For positive control, AGS is treated by 1 ml of *H. pylori* suspension. For the measurement of suppression of attachment, AGS is treated by 1 ml of *H. pylori* suspension and *Pediococcus acidilactici* J9 (200ug/ml). The culture plates seeded with AGS treated by H. pylori and *Pediococcus acidilactici* J9 are incubated for 90 minutes at 37°C in a 5% CO_2 incubator. The culture media is eliminated and the cells are carefully harvested. The cells are suspended in 500 ul of PBS then examined with FACS.

Statistical Analysis

All values shown in the figures are presented as mean ± standard error. Western blot results were analyzed by Student's *t*-test. A 2-tailed probability value below 0.05 was considered statistically significant. Data were analyzed using SPSS version 17.0 (SPSS Inc., USA).

Declarations

Ethics approval and consent to participate

All experimental animal procedures performed were approved by the Institutional Animal Care and Use Committee (IACUC, Approval number: 16-0043-C2A1) of Seoul National University Hospital, which was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported from the 0720142100 University Industrial Technology Force.

Authors' contributions

ML performed experiments, analyzed data, wrote paper, JC provided advice in study design, critically discussed results, co-edited paper, KYK provided advice in study design, critically discussed results, co-edited paper, WI provided advice in study design, critically discussed results, co-edited paper, MK provided advice in study design, critically discussed results, co-edited paper.

Acknowledgements

The authors gratefully acknowledge Financial assistance from the 0720142100 University Industrial Technology Force. We also express our appreciation to professor Nayoung Kim from Seoul National University Bundang Hospital for kindly providing *H.pylori* stock and the human gastric adenocarcinoma cell lines AGS (KCLB 21739; Korea) cells.

References

1. Cross ML, Ganner A, Teilab D, Fray LM. Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria. FEMS Immunol Med Microbiol. 2004;42:173–80.
2. Jack RW, Tagg JR, Ray B. Bacteriocins of gram-positive bacteria. Microbiol Rev. 1995;59:171–200.

3. De Vuyst L, Leroy F. Bacteriocins from lactic acid bacteria: production, purification, and food applications. J Mol Microbiol Biotechnol. 2007;13:194–9.
4. Josson K, Scheirlinck T, Michiels F, Platteeuw C, Stanssens P, Joos H, et al. Characterization of a gram-positive broad-host-range plasmid isolated from Lactobacillus hilgardii. Plasmid. 1989;21:9–20.
5. Marshall BJ. Helicobacter pylori. Am J Gastroenterol. 1994;89:116-28.
6. Lind T, Megraud F, Unge P, Bayerdorffer E, O'Morain C, Spiller R, et al. The MACH2 study: role of omeprazole in eradication of Helicobacter pylori with 1-week triple therapies. Gastroenterology. 1999;116:248–53.
7. Maeda S, Yoshida H, Matsunaga H, Ogura K, Kawamata O, Shiratori Y, et al. Detection of clarithromycin-resistant helicobacter pylori strains by a preferential homoduplex formation assay. J Clin Microbiol. 2000;38:210–4.
8. Maeda S, Yoshida H, Ogura K, Kanai F, Shiratori Y, Omata M. Helicobacter pylori specific nested PCR assay for the detection of 23S rRNA mutation associated with clarithromycin resistance. Gut. 1998;43:317–21.
9. Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y. Lactic acid-mediated suppression of Helicobacter pylori by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model. Am J Gastroenterol. 1998;93:2097–101.
10. Ierardi E, Giorgio F, Losurdo G, Di Leo A, Principi M. How antibiotic resistances could change Helicobacter pylori treatment: A matter of geography? World J Gastroenterol. 2013;19:8168–80.
11. Sachdeva A, Nagpal J. Effect of fermented milk-based probiotic preparations on Helicobacter pylori eradication: a systematic review and meta-analysis of randomized-controlled trials. Eur J Gastroenterol Hepatol. 2009;21:45–53.
12. Hsieh PS, Tsai YC, Chen YC, Teh SF, Ou CM, King VA. Eradication of Helicobacter pylori infection by the probiotic strains Lactobacillus johnsonii MH-68 and L. salivarius ssp. salicinius AP-32. Helicobacter. 2012;17:466–77.
13. Johnson-Henry KC, Nadjafi M, Avitzur Y, Mitchell DJ, Ngan BY, Galindo-Mata E, et al. Amelioration of the effects of Citrobacter rodentium infection in mice by pretreatment with probiotics. J Infect Dis. 2005;191:2106–17.
14. Tien MT, Girardin SE, Regnault B, Le Bourhis L, Dillies MA, Coppee JY, et al. Anti-inflammatory effect of Lactobacillus casei on Shigella-infected human intestinal epithelial cells. J Immunol. 2006;176:1228–37.
15. Fernandez MF, Boris S, Barbes C. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol. 2003;94:449–55.
16. Lionetti E, Indrio F, Pavone L, Borrelli G, Cavallo L, Francavilla R. Role of probiotics in pediatric patients with Helicobacter pylori infection: a comprehensive review of the literature. Helicobacter. 2010;15:79–87.
17. De Keersmaecker SC, Verhoeven TL, Desair J, Marchal K, Vanderleyden J, Nagy I. Strong antimicrobial activity of Lactobacillus rhamnosus GG against Salmonella typhimurium is due to accumulation of lactic acid. FEMS Microbiol Lett. 2006;259:89–96.
18. Sartor RB. Probiotic therapy of intestinal inflammation and infections. Curr Opin Gastroenterol. 2005;21:44–50.
19. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. Am J Physiol. 1999;276:G941-50.

20. Coconnier MH, Lievin V, Hemery E, Servin AL. Antagonistic activity against Helicobacter infection in vitro and in vivo by the human Lactobacillus acidophilus strain LB. Appl Environ Microbiol. 1998;64:4573–80.
21. Mehta R, Arya R, Goyal K, Singh M, Sharma AK. Bio-preservative and therapeutic potential of pediocin: recent trends and future perspectives. Recent Pat Biotechnol. 2013;7:172–8.
22. Sipponen P, Hyvarinen H. Role of Helicobacter pylori in the pathogenesis of gastritis, peptic ulcer and gastric cancer. Scand J Gastroenterol Suppl. 1993;196:3–6.
23. Chiba N, Rao BV, Rademaker JW, Hunt RH. Meta-analysis of the efficacy of antibiotic therapy in eradicating Helicobacter pylori. Am J Gastroenterol. 1992;87:1716–27.
24. Andersson DI, Hughes D. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol. 2010;8:260–71.
25. Kong YH, Zhang L, Yang ZY, Han C, Hu LH, Jiang HL, et al. Natural product juglone targets three key enzymes from Helicobacter pylori: inhibition assay with crystal structure characterization. Acta Pharmacol Sin. 2008;29:870–6.
26. Preidis GA, Versalovic J. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. Gastroenterology. 2009;136:2015–31.
27. Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, et al. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology. 2001;121:580–91.
28. Tannock GW. Probiotic properties of lactic-acid bacteria: plenty of scope for fundamental R & D. Trends Biotechnol. 1997;15:270–4.
29. Dickerson FB, Stallings C, Origoni A, Katsafanas E, Savage CL, Schweinfurth LA, et al Effect of probiotic supplementation on schizophrenia symptoms and association with gastrointestinal functioning: a randomized, placebo-controlled trial. Prim Care Companion CNS Disord 2014;16.
30. Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. Microencapsulation of probiotics for gastrointestinal delivery. J Control Release. 2012;162:56–67.
31. Yan F, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem. 2002;277:50959–65.
32. Matsumoto M, Tsuji M, Sasaki H, Fujita K, Nomura R, Nakano K, et al. Cariogenicity of the probiotic bacterium Lactobacillus salivarius in rats. Caries Res. 2005;39:479–83.
33. Geier MS, Butler RN, Howarth GS. Probiotics, prebiotics and synbiotics: a role in chemoprevention for colorectal cancer? Cancer Biol Ther. 2006;5:1265–9.
34. Floch MH. Probiotics. Irritable Bowel Syndrome, and Inflammatory Bowel Disease. Curr Treat Options Gastroenterol. 2003;6:283–8.

Figures

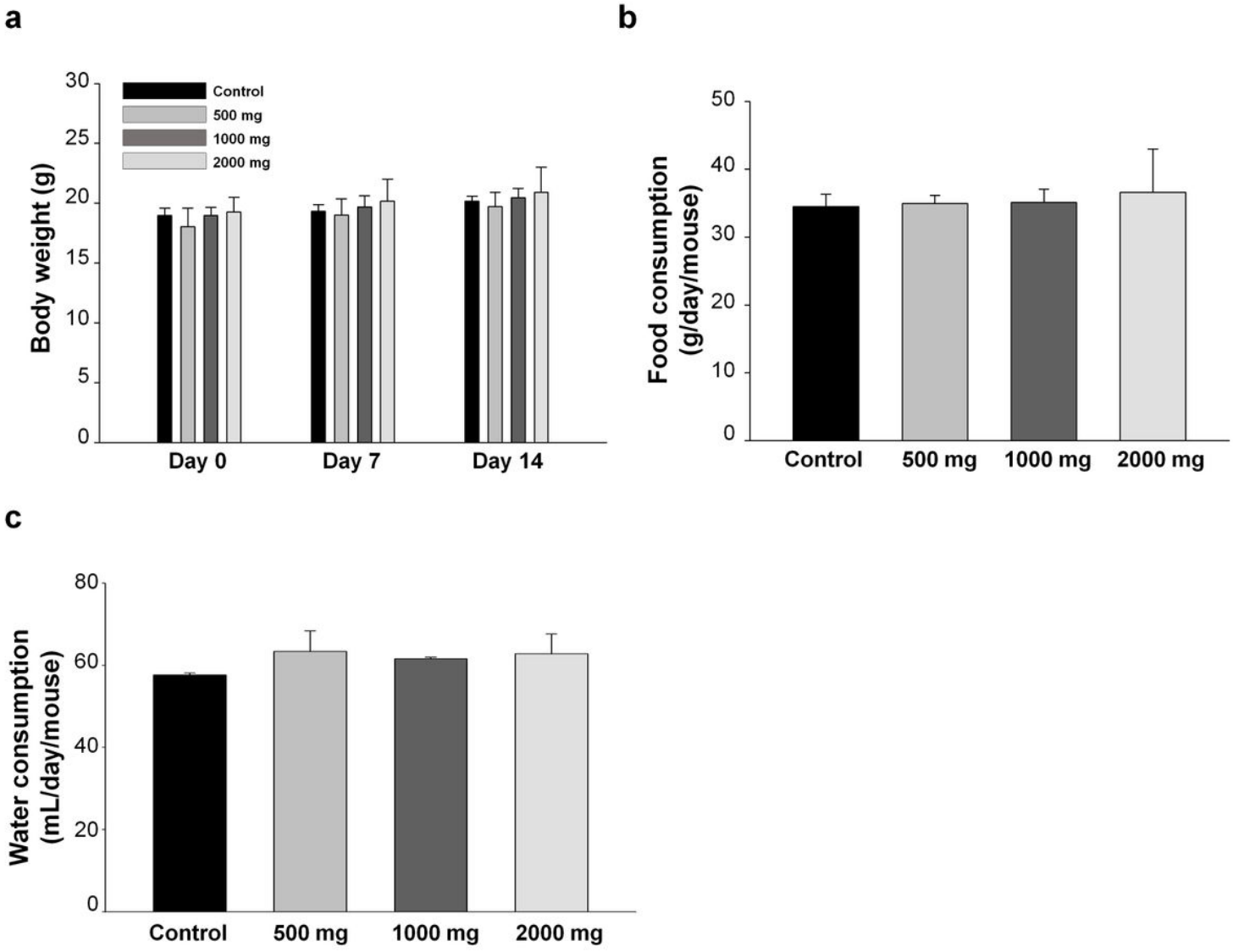

Figure 1

Changes in body weight and intake of C57BL/6 mice which treated Pediococcus acidilactici J9. Dosage is set by the standard of MFDS. Maximum dosage is set to 2,000 mg/kg/day for both male and female, and with the geometric ratio of 1/2, low dose group, medium dose group, and high dose group are set by per body weight (kg) respectively. a For every animal, change of weight is measured just before the administration of test materials once a week at certain time during 2 weeks. b, c Intake of feeds and water is measured and calculated once a week. Feeds; solid feeds for laboratory animal are freely offered, and drinking water; filtration-purified water is also freely offered. N = 10 samples per group.

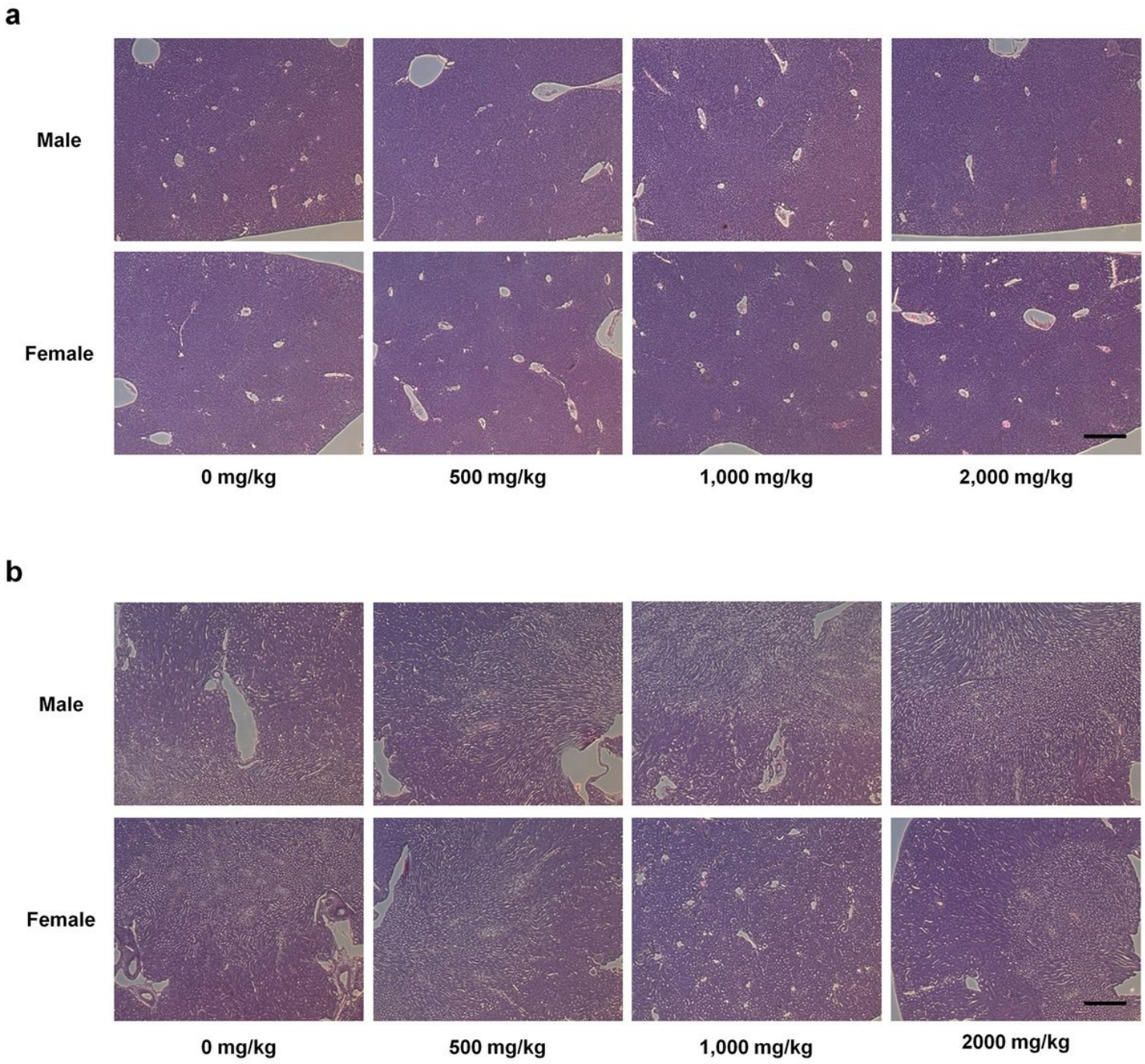

Figure 2

Histopathological examinations of the liver and Kidney. a, b Female and male C57BL / 6 mice were orally administered with Pediococcus acidilactici J9 for 14 days. The liver and kidneys of the control and Pediococcus acidilactici J9 administration animals were extracted and fixed with 10% neutral buffered formalin solution on the final autopsy day of all animals after gross lesion observation. Pathological lesions and structures such as liver, kidney infection, necrosis, and iron and bilirubin pigmentation were confirmed by H & E staining. Bar = 30μm, N = 10 samples per group.

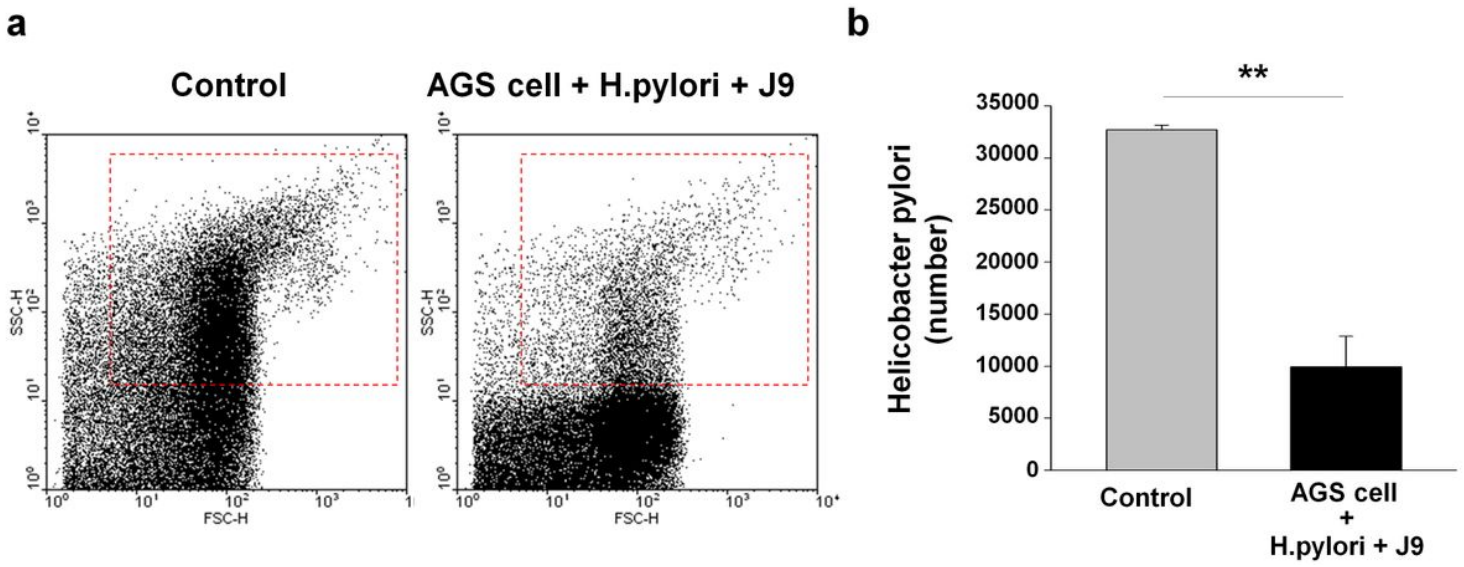

Figure 3

Pediococcus acidilactici J9 inhibits adhesion and growth of H. pylori in gastric epithelial cells. a After H. pylori supernatant and Pediococcus acidilactici J9 at 200 ug / ml concentration were treated for 24 hours in AGS cells, H.Pylori count was confirmed by flow cytometry. In the control group, the H.pylori number was confirmed by flow measurement after 24 h of treatment with H.pylori in the AGS cell. b H. pylori number was quantified by a flow cytometer. The experiment was repeated three times and the data are shown as the mean (SEM). **p<0.01 versus control group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx