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ORIGINAL ARTICLE

Probiotic Bacteria *Bacillus Licheniformis* Attenuates Gastric Ulcer Healing and Antagonizes the Ulcer Healing Effect of Ranitidine in Rats

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ABSTRACT
Bacillus licheniformis is widely used as probiotic. It is reported to potentiate the effect of conventional antiulcer therapy in humans. The effect of Bacillus licheniformis on gastric ulcer healing and its interaction with ranitidine, a known antiulcer drug, was studied using acetic acid induced chronic gastric ulcer model in rats. The bacteria was administered orally at two different doses of 10^6 cells/kg and 10^3 cells/kg while ranitidine was given at a dose of 50 mg/kg. Bacillus licheniformis attenuated the ulcer healing in rats. It increased the ulcer score and ulcer index compared to vehicle treated animals. Histological studies of the ulcerated tissues supported the macroscopic findings. As expected, ranitidine increased gastric ulcer healing while the combination of ranitidine and Bacillus licheniformis showed decreased ulcer healing compared to ranitidine alone. It was concluded that Bacillus licheniformis aggravates gastric ulcer and antagonizes the effect of ranitidine on ulcer healing in rats.
Keywords: Bacillus licheniformis; Acetic acid; Gastric ulcer; Ranitidine; Probiotic

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INTRODUCTION

Probiotics are viable microbes that when consumed in specific numbers are known to possess beneficial effects on the health and well being of the host beyond that provided by basic nutrition [1]. The probiotics are known to reduce/prevent diseases of the upper digestive tract. One of the most commonly reported beneficial effect of probiotics is on the peptic ulcer disease. Several probiotics from *Lactobacillus* species are reported to augment the gastric ulcer healing through eradication of *H pylori,* the organism responsible for development of peptic ulcers [2]. Other probiotics that are reported to eradicate *H pylori* from stomach includes *Pediococcus pentosaceus, Bifibacterium bifidus* and *Bacillus lechiniformis* [2]. Furthermore, *Lactobacillus gasseri* OLL 2716 is reported to increase healing of acetic acid induced gastric ulcers in rats [3].

Apart from this, several other probiotics are known to reduce gastric ulcer formation independent of *H pylori* infection [4-8]. Though probiotics are generally considered as safe, some of the recent reports suggest that their consumption may produce harmful effects on the host. One of the probiotics that is reported for both beneficial and toxicogenic effect is *Bacillus lechiniformis*. It is a Gram-positive rod bacteria included in the 'subtilis' group that is used as probiotic in humans and pigs [9,10]. Its used as probiotic by humans has remained controversial with some authors reporting beneficial effects [11,12], while a majority of the published reports including those from WHO recommend not to use this bacteria as probiotic [13-15] and there are also few reports that claim this bacteria can contribute to antiobiotic resistance [16]. This probiotic is widely used for human consumption in Russia, Ukraine and China [16].

One of the beneficial effects reported for these bacteria is on the gastric ulcer. Previous study from China indicates *Bacillus licheniformis* treatment along with standard anti-*H. pylori* treatment can reduce peptic

ulcer in patients more effectively than triple therapy alone consisting of lansoprazole (antiulcer drug), amoxicillin (antibiotic) and levofloxacin (antibiotic) while it does not alter the ulcer healing effect of quadruple therapy consisting of bismuth pectin (antiulcer drug), lansoprazole (antiulcer drug), (antibiotic) and levofloxacin (antibiotic) significantly [17]. To explore the effect of this probiotic on gastric ulcer healing independent of *H. pylori* eradication, the present study was carried out to determine its effect on the ulcer healing in experimentally induced acetic acid induced chronic gastric ulcers in rats. The pharmacodynamic interaction between *Bacillus licheniformis* and ranitidine was also determined to study the effect of co-administration of *Bacillus licheniformis* with antiulcer agents.

MATERIALS AND METHODS

Materials: The *Bacillus licheniformis* was purchased from a supplier in China, where these bacteria are used as probiotic. Capsules containing 25 million bacteria per capsule were procured. The bacteria were subcultured in nutrient broth followed by separation through centrifugation. The cell number was adjusted to either 10^6 or 10^3 cells per ml using McFarland's standard followed by dilution. The animals were administered with these bacteria orally at a dose of either 10^6 or 10^3 cells per kg body weight once daily. The dose was selected from daily human dose using the conversion formula [18].

Animals: Male Wistar albino rats weighing between 180-210 g were used. The animals were maintained under standard conditions of 12:12 h light dark cycle at a temperature of 25±2 ^{0}C. The experimental protocol was approved by the research committee of the institute for its ethical and scientific content.

Acetic acid-induced chronic gastric ulcer: The method reported by us earlier was followed [19]. The animals were fasted for 24 h prior to the experiment. Under light ether anesthesia, the abdomen was opened by midline incision below the xiphoid process and the stomach was exposed. Glacial acetic acid (0.05 mL) was applied onto the serosal surface using cylindrical mould (6 mm), which was allowed to remain there for 60 s. The acid solution was then removed by rinsing the mold with 0.9% saline to prevent possible damage to the surrounding tissues close to the point of application. The stomach was placed back carefully and the abdominal wall was closed. The animals were divided into six different groups; the first group served as control while the group 2 received ranitdine (50 mg/kg, p.o). The third and fourth group rats were treated with *Bacillus licheniformis* at dose of 10^6 cells/kg and 10^3 cells/kg respectively. The last two groups received combination of *Bacillus licheniformis* (10^6 cells/kg) with ranidine (50 mg/kg, p.o) and *Bacillus licheniformis* (10^3 cells/kg) with ranidine (50 mg/kg, p.o) respectively. Rats were killed 6 h after the last dose, stomach was removed and was cut open along the greater curvature. The total mucosal area and total ulcerated area were measured. The ulcer index was determined using the formula:

$$\text{Ulcer index} = \frac{10}{X}$$

where X = total mucosal area/total ulcerated area.

The ulcers were assigned scores based on the intensity as follows; 0= no ulcer, 1=superficial lesion, 2= deep or penetrated ulcer and 3= perforated ulcer wherein the stomach is supported by surrounding tissues.

The ulcerated tissue was subjected to histopathological studies to determine the effect on surface epithelial tissue. The liver and kidney sections were also taken to determine the effect of treatments on these organs.

Statistical Analysis

Values are expressed as mean + standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's test for comparison of all parameters. Statistical significance for ulcer score was determined using Kruskal-Wallis test with post test. The statistical analysis was done using computer software (Graphpad lnstat DATASET *1,* ISD, software version 3.0 for Windows). Values of $p<0.05$ were considered to indicate statistical significance.

RESULTS

The glacial acetic acid application onto the serosal surface of the stomach produced penetrating ulcers in the control group. The ulcer were deep with very less epithelium as compared to stomach from normal animals (Fig. 1 and Fig. 2).

Ranitidine increased the ulcer healing as indicated by reduction in ulcer score and ulcer index (Table 1). Histological examination of the stomachs from ranitidine treated animals revealed regeneration of surface epithelium indicating healing of ulcers (Fig. 3).

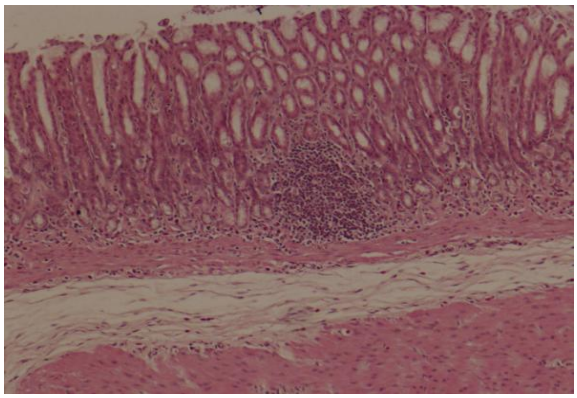

Fig 1: Stomach from normal animals showing mucosa, submucosa and muscularis layers.

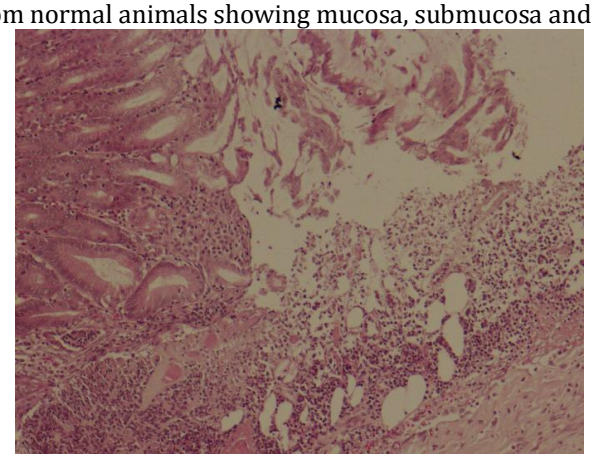

Fig 2: Stomach from control group rat, wherein there is no surface epithelium.

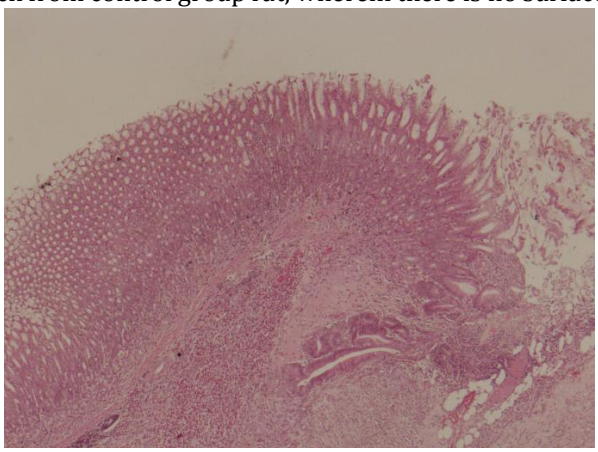

Fig 3: Stomach from ranitidine treated rat showing regenerated surface epithelium.

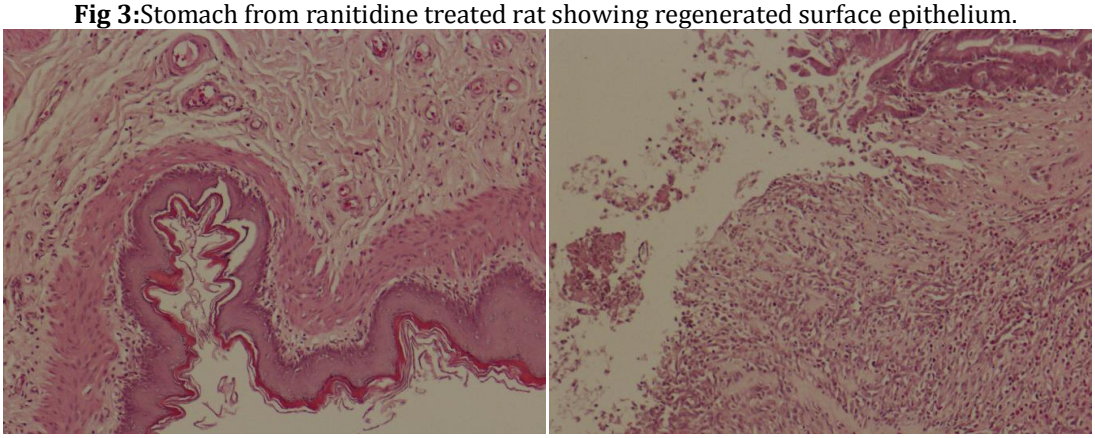

Fig 4 and Fig 5: Stomach tissue from *Bacillus licheniformis* treated animals showing complete absence of epithelium. Right- *Bacillus licheniformis* 10^3 cells/kg; Left - *Bacillus licheniformis* 10^3 cells/kg

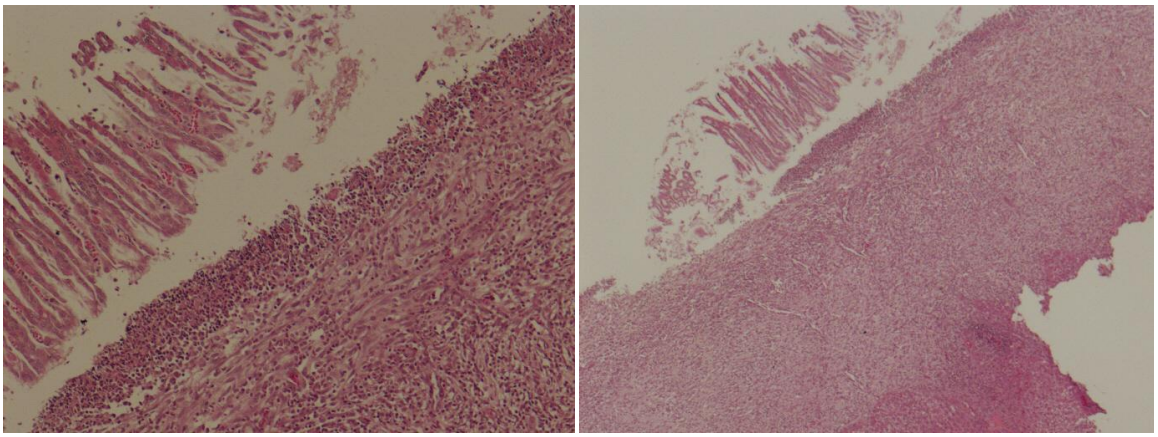

Fig 5 and Fig 6: Stomach tissue from *Bacillus licheniformis* + ranitidine treated animals. There is small amount of epithelium similar to control group. Right- *Bacillus licheniformis* 10^3 cells/kg+ ranitidine; Left - *Bacillus licheniformis* 10^3 cells/kg + ranitidine.

Treatment with the bacteria; *Bacillus licheniformis* at both doses (10^6 cells/kg or 10^3 cells/kg) decreased the ulcer healing compared to the control group. The ulcerated area was more that led to a significant increase in ulcer index when compared to the control group. The ulcers were deep and ulcer score was significantly more in *Bacillus licheniformis* (10^6 cells/kg) treated group compared to control group. The macroscopic results were supported by histological findings, wherein, stomach of animals treated with *Bacillus licheniformis* did not had any surface epithelium and tissue underlying the epithelium. Complete absence of epithelium and underlying structures indicates penetrating ulcers in these group of animals (Fig 4 and Fig 5).

The co-administration of ranitidine with *Bacillus licheniformis* showed decreased ulcer healing compared to ranitidine treatment alone. There was no significant difference in ulcer index between control group and the group that received ranitidine with either dose of *Bacillus licheniformis* indicating that these bacteria antagonizes the ulcer healing effect of ranitidine. A similar effect was observed with ulcer score wherein ranitidine treatment reduced the ulcer score when compared to control and treatment of ranitidine along with *Bacillus licheniformis* did not show any significant effect when compared to control (Table 1). Histological examination of the ulcerated tissues from animals of these groups showed that surface epithelium was completely missing in stomach of animals and tissue resembled those from the control group (Fig 6 and Fig 7).

Table 1: Effect on healing of acetic acid induced chronic gastric ulcers in rats.

Groups	Ulcer index	Ulcer score	Mortality
Control	0.39±0.010	2.50±0.223	0/6
Ranitidine (50 mg/kg, *p.o*)	0.09±0.013***	0.16±0.166*	0/6
Bacillus licheniformis (10^3 cells/kg, *p.o*)	0.65±0.014***	3.00±0.00	1/7
Bacillus licheniformis (10^6 cells/kg, *p.o*)	0.73±0.012***	2.50±0.224^{+}	2/8
Bacillus licheniformis (10^3 cells/kg, *p.o*) + Ranitdine (50 mg/kg, *p.o*)	0.42±0.011^{+++}	2.50±0.224^{+}	2/8
Bacillus licheniformis (10^3 cells/kg, *p.o*) + Ranitdine (50 mg/kg, *p.o*)	0.49±0.013^{+++}	2.50±0.224^{+}	2/8

All values are mean±SEM, n=6, *P<0.05, ***P<0.001 compared to control, $^{+}$P<0.05, $^{+++}$P<0.001 compared to ranitidine $^{\#\#\#}$P<0.001 compared to *Bacillus licheniformis* alone

DISCUSSION

In the present study, administration of probiotic *Bacillus licheniformis* reduced healing of ulcer in rats. This is contrary to the belief that these bacteria may be beneficial in the treatment of gastric ulcers.

As mentioned earlier, *Bacillus licheniformis* is reported to potentiate the effect triple antiulcer therapy used in eradication of *H. pylori,* the microorganism responsible for development of ulcers in humans[17]. Though this study was done using patients, it failed to explain the mechanism responsible for potentiating effect of *Bacillus licheniformis*. The probable mechanism explained by authors was that *Bacillus licheniformis* may compete with *H pylori* for nutrients resulting in faster eradication of *H pylori.* There was

no mention of effect of ulcers independent of *H pylori* infection, which account for significant number of ulcer cases in humans.

In the present study, the effect of *Bacillus licheniformis* on *H pylori* independent ulcers was studied. There are several reports on the effect of other bacteria on *H pylori* independent ulcers. Earlier reports on the influence of administration of microbes on gastric ulcer healing reveals that microorganisms affects healing of acetic acid induced chronic gastric ulcers [3,8]. Induction of *Lactobacillus* along with administration of antibiotics was reported to suppress colonization of gram-negative bacteria and increase healing of acetic acid induced gastric ulcers20. However, inoculation of *Candida albicans* is reported to delay ulcer healing and attenuate the ulcer healing effect of *Lactobacillus*8. Furthermore, only *Lactobacillus* is also reported to reduce the development of non-steroidal anti-inflammatory drugs (NSAIDs) induced gastric ulcer in rats4. This effect has also been attributed to the suppression of gram-negative bacteria such as *Escherichia coli, Klebsiella* and *Proteus* indicating that bacterial influence on gastric ulcer differs from normal antiulcer drugs, that reduce gastric acid and/or increase gastric mucus secretion. It is worth mentioning here that only viable bacteria have been reported to affect gastric ulcers and no effect was observed with γ-ray radiated *Lactobacillus* [3].

The antibacterial effect reported for *Bacillus licheniformis* has also been contradicted. An *in-vivo* study in gnotobiotic mice reported that *Bacillus licheniformis* does not alter the growth of any of bacteria, whose growth were reported to be prevented by this probiotic in *in-vitro* studies [21]. The biochemical functions of *Bacillus licheniformis* are different from those that are observed with other probiotics. The biochemical changes observed in gnotobiotic mice after *Bacillus licheniformis* were very mild and the utilization of indigenous changes in enzymes and fatty acid were different from those seen with normal probiotics indicating that *Bacillus licheniformis* may not have all the attributes of a typcial probiotic [10].

The use of *Bacillus licheniformis* has always been controversial. Many authors recommend avoiding use this bacteria as probiotic [22]. Furthermore, administration of *Bacillus licheniformis* is reported to be associated with bacteremia and septicemia, endocarditis, meningitis, and infection of wounds in ears, eye, respiratory tract, urinary tract and gastrointestinal tract [23]. Moreover, the use of *Bacillus licheniformis* is associated with antiobiotic risk and its use for humans is not recommended due to this effect [16].

To conclude, *Bacillus licheniformis* reduces gastric ulcer healing and antagonizes the effect of ranitidine on ulcer healing in rats. The effect is dose-dependent at doses equivalent to those used in humans.

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