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Suppression of Helicobacter Pylori in Mice Intestine by Ingestion of Lactic Acid Bacteria in Yoghurt.

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Abstract: *Helicobacter pylori (H. pylori) is a major cause of chronic gastritis and peptic ulcer. Antibiotics-based H. pylori eradication treatment is 90% effective, but it is expensive and can have side effects and antibiotic resistance. Probiotics costs less and are easily available. These studies were conducted to investigate the role of probiotics in the treatment of H. pylori infection. H. pylori were recovered from stool culture of peptic ulcer patients. Twenty albino wister mice weighing (25g –29g) were kept in 5cages, A to E (4 mice per cage). Faecal occult blood (FOB) test and viable bacterial count (VBC) were done on all the mice at different stages of the experiment. Animals in cages A to D were fed orally with different concentrations of H. pylori for 1week. Animals in cage E were fed with water as control. Finally the test mice were fed for another one week with yogurt which contains lactic acid bacteria then a retest was done. The results generated showed that the H.pylori can induce stomach ulcer and the Lactic acid bacteria in the yogurt suppressed the ulcer disease. Conclusively, this study has proven that Lactic acid bacteria in yoghurt can suppress Helicobacter pylori infection in mice.*

Key words: *Helicobacter pylori, lactic acid bacteria, peptic ulcer, yoghurt, probiotics,*

I. Introduction

Helicobacter pylori (H. pylori) is a gram-negative, microaerophilic bacterium that inhabits the gastric mucosa and various areas of the stomach and duodenum. It was identified in 1982 (1). It has been established that H. pylori infection is a major cause of chronic gastritis and peptic ulcer disease (2). H. pylori is the causative agent for acute and chronic gastritis and gastric lymphoma. The organism has 2 to 6 unipolar, sheathed flagella of approximately 3μm in length. The flagella confer motility and allow rapid movement in viscous solutions such as the mucus layer overlying the gastric epithelial cells (3).

The prevalence of H. pylori infection varies widely by geographic area, age, race, and socioeconomic status. In the adult population of industrialized countries, it's prevalence is estimated to be at 20–50%, while in the developing countries, the rate is as high as 80% (4). H. pylori infection rates rise rapidly in the first 5 years of life indicating that H. pylori is acquired early in childhood (5). It has been proposed that Helicobacter strains could be part of the indigenous microbiota of the human stomach and that H. pylori could have both pathogenic and symbiotic features (6). H. pylori colonizes the host's gastric epithelium(7). The colonization of the mucosa evokes local inflammatory responses, which result in further mucosal injury but are not able to clear the infection (8). H. pylori infection thus escapes the natural gastrointestinal defenses, which allows it to induce chronic infection due to it's virulence factors such as urease and cytotoxin which induces strong inflammatory response in the gastric mucosa and results in epithelial cell damage (9). In the absence of treatment, chronic H. pylori infection persists for years(10).

In 1994, the National Institutes of Health (USA) recommended eradication treatment of H. pylori consists of a combination of two antimicrobials and an acid-suppressive drug (11). A triple treatment was initiated but that also had some shortcomings, such as several adverse effects possibly leading to discontinuation of the treatment and limited efficacy particularly because of antimicrobial resistance (12). However, treatment of infection is challenged by the rapid rate with which the bacteria acquire resistance to the drugs, poor compliance, an excessively high bacteria load, impaired mucosal immunity, early re-infection and the presence of intracellular bacteria (13).

In recent years, the development of alternative anti-H. pylori treatments has been pursued, and investigations have been carried out to define components that could be used either as monotherapy in combination with antimicrobials thus resulting in more effective anti-H. pylori therapy or alternative ways of controlling H. pylori infection. These novel treatments could potentially reduce the costs related to the treatment of H. pylori associated diseases. Promising results have been obtained in initial studies with several probiotic strains. Probiotics have been proven to be useful in the treatment of several gastrointestinal diseases such as acute

infectious diarrhea (14). According to (15) probiotics are live microorganisms (such as Lactobacillus spp. and Bifidobacterium spp.), that, when administered in adequate amounts confer a health benefit beyond inherent basic nutrition on the host. (16) inferred that for bacteria to give their probiotic effects, they must be able to tolerate the intestinal acidic P^H and the bile acids of intestine. The intake of probiotics can be beneficial in H.pylori infected subjects. According to (17), probiotics are complementary to antibiotics and may have the potential to reduce the adverse effects of triple anti-Helicobacter treatment so as to improve the eradication rate. (18) reported that probiotic therapy is generally fast, safe and cost effective hence the aim of the present study, to investigate the effects of probiotics in H. pylori infected subjects receiving the recommended dosage of lactic acid bacteria in yoghurt, to evaluate if the infection could be suppressed using mice as animal model.

II. Materials And Methods

Twenty laboratory reared albino wister mice were purchased. They were divided into five cages 4mice to a cage. Their weights were taken and recorded. The cages were labelled A to E. Cages A to D contained the test animals while cage E contained the control animals. Occult blood test using Acon Faecal Occult blood test strip and viable bacterial count of their stool samples were done according to the method of (19). Cultural and biochemical methods for the isolation of H. pylori by (20) were adopted for isolation of H. pylori from ulcer patients. Animals in cages A to D were fed orally with different doses of H. pylori 0.2mg, 0.4mg, 0.6mg, and 0.8mg respectively for 1week, while animals in cage E were fed with only water and after which another occult blood test and viable bacterial count were done on their stool samples.

The animals were then fed with different grades of lactic acid bacteria in yoghurt for 1week except the animals in cage E, after which occult blood test and viable bacterial count were done on their stool samples. Their final weights were also taken.

III. Result

There were no obvious differences in the morphology of the stool of the mice except that the number of their stool per day increased during H. pylori feeding while it decreased in number during Yoghurt feeding.
The weight of the test mice increased more than the control mice after the yoghurt meal, this is shown in table 1.
The viable bacterial count done on the stool of the mice showed an increase in the bacterial counts when they were fed with helicobacter pylori but this count decreased when they were fed with lactic acid bacteria, this is shown in table 2.

The occult blood tests done on the stool of mice at the onset of the experiment were negative but when the mice were retested after the H.pylori feeding, the result gotten were positive and after the lactic acid yoghurt feeding, the retest for the occult blood showed negative results, this is shown in table 3. The control mice which were fed with feed and water only, tested negative all through.

TABLE 1.Initial and final weight of mice and the concentration of the different bacteria administered to the mice.

S/N	No of Mice	Weight before yogurt	Concentration of H. pylori	Concentration of Lactic acid	Weight after experiment	experiment	bacteria in
Cage A	4	29g each	0.2mg		0.2mg	30g, 32g, 32g, 33g	
Cage B	4	25g each	0.4mg	0.4mg	27g, 27g, 28g,27g		
Cage C	4	27g each	0.6mg	0.6mg	28g, 27g, 29g, 29g		
Cage D	4	28geach	0.8mg		0.8mg	30g, 29g 31g, 30g	
Cage E (control)	4	27g each	only water	0.0mg	29g, 29g, 28g, 28g		

TABLE 2.Viable count of bacterial from stool of mice before and after the different bacteria feeding using the reading from the highest dilution (10^4)

S/N	Count before feeding	Count after H.pylori feeding	Count after Lactic acid yogurt feeding
	mice 1, 2, 3, 4	mice 1, 2, 3, 4	mice 1, 2, 3, 4
Cage 1	104,122, 121,118	169, 197, 200,164	156, 142, 167, 166
Cage 2	164,171,143, 118	232,200,194, 251	200, 182, 195, 193
Cage 3	104,187, 123,158	132,232,160,192	118, 189, 122, 154
Cage 4	88, 73, 123, 144	132,111,142, 201	132, 97, 100, 189
Cage 5(control)	118, 165, 89, 79	177, 173, 67, 88	178, 154, 86, 98

TABLE 3. The result of the feacal occult blood test done on the mice at different stages of the work

S/N	Occult blood tests result before bacteria	Occult blood tests result after	Occult blood tests result after

	feeding.	H.pylori feeding.	Lactic acid yogurt feeding.
	Mice 1, 2, 3,4	Mice 1, 2, 3,4	Mice 1 2, 3, 4
Cage 1	Negative	Positve	Negative
Cage 2	Negative	Positve	Negative
Cage 3	Negative	Positve	Negative
Cage 4	Negative	Positve	Negative
Cage5 controls	Negative	Negative	Negative

IV. Discussion

In this study albino wister mice were used to assess the suppressive effect of Lactic acid bacteria (LAB) on H. pylori.

Results showed that Lactic acid bacteria yoghurt had better effect on H. pylori infection according to the short duration of the treatment. Viable counts of bacteria showed increase in numbers of bacteria count after the H. pylori feeding which was reduced by the presence of Lactic acid bacteria (LAB) in the yoghurt. This is in line with the work of (21) who reported that, as the amount of lactic acid increased, the number of H. pylori decreased because lactic acid bacteria inhibit the growth and urease activity of H. pylori. In another work, (22) reported that administration of fermented milk containing the strain of L. acidophilus La1 decreased H. pylori urease activity.

The frequent defecating seen in the test mice showed that the H. pylori is causing gastrointestinal disturbances or inflammation of the stomach in the mice. This is in line with the work of (23) who reported that H. pylori-induced inflammation of the stomach.

There was also an increase in their weight after feeding the mice with yoghurt. This could be attributed to the reduction of gastric inflammation which brought about reduced frequency in defecating which was reported by (24) who found Lactobacillus gasseri to be effective in suppression of H. pylori infection and reduction in gastric mucosal inflammation hence allowing the food taken in to be well absorbed and utilized by the mice for body building.

This study has demonstrated that H. pylori can actually cause stomach ulcer in laboratory animals within a very short period of time and even at little concentration. The study also shows that Lactic acid bacteria in yoghurt can serve as a curative measure for the stomach ulcer by increasing the acidity of the intestine and inhibiting the reproduction of many harmful bacteria as recorded by (25).

In conclusion, this study showed that probiotics have positive effect on the suppression of H. pylori infection as indicated by Acon Faecal Occult blood test strip. Probiotic is recommended in order to suppress H. pylori and to reduce the frequency of antibiotic induced side effects during treatment with antibiotic

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