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Demonstrating the Clinical Efficacy of Certain Probiotic Strains in Acute and Chronic Diarrhea

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ABSTRACT

The present study was conducted to demonstrate the clinically efficacy of few probiotic strains freeze dried in the laboratory in acute and chronic diarrhea. The strains used in the study were isolated from commercially available probiotics and identified in the laboratory and finally subjected to freeze drying in pure cultures. After freeze drying these probiotics were filled in capsule aseptically and given to the patients with acute and chronic diarrhea to demonstrate their clinical effectiveness. This was a blind placebo control study which included healthy volunteers as well. The effectiveness of the treatment was assessed by the recovery of the ailment and improvement of the symptoms associated with the illness. Dehydrated skimmed milk was used as placebo. From the result of clinical studies it can be concluded that probiotics make a significant alternate treatment to control acute diarrhea and improve chronic diarrhea.

Keywords: Probiotcs, acute and chronic diarrhea, strains, skimmed milk and placebo

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INTRODUCTION

The dietary use of live microorganisms has a long history. Soured milks and cultured dairy products, such as kefir**,** koumiss, leben and yogurt were often used therapeutically before the existence of microorganisms was recognized[1]. The term Probiotic was used by[2]Fuller in 1989 to indicate "live microbial food supplement" which beneficially affects the host by improving the intestinal microbial balance. More recently, the meaning of this word has been refined several times and today a widely accepted definition of probiotics is: "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host"[3]. Less-established evidence suggests that certain bacteria may provide an alternative to antibiotics[4]. Our knowledge of the benefits associated with probiotic bacteria dates back to the beginning of the previous century when the Nobel Prize laureate, the Russian-bornElie Metchnikoff, intrigued by the longevity of the Caucasian population and its frequent consumption of fermented milks developed the theory that "The presence of lactic acid bacteria in the intestine can control infections caused by pathogenic microorganisms and can help to control toxin-producing bacteria. The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". He developed and prescribed to his patients bacterio therapy, i.e. the use of lactic acid bacteria in dietary regimens[5].This describes in a clear way the "Probiotic Concept". He spoke highly about the possible health benefits of the lactic acid-bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*[6] Another researcher involved in this field was by Henry who discovered *Bifidobacteria* in the feaces of healthy, breastfed infants and was the first researcher who started promoting their therapeutic properties. He believed that when *Bifidobacteria* displace putrefactive bacteria in the intestines, the correct microbial balance is restored. The evidence that microbial colonization has important health implications was first shown in a study that compared germ-free animals[7]. [8]The study concluded that the germ-free animals were much more susceptible to infection. It is hypothesized that the beneficial bacteria may impede the growth of pathogenic bacteria in the following ways: by "crowding" them out, by producing antimicrobial substances that directly inhibit pathogens, and by releasing hydrogen perioxide, a bactericidal agent [9]. Although Metchnikoff's ideas were clearly related to lactic acid bacteria in dairy products, the interest of other scientists soon turned to lactic acid bacteria of intestinal origin. One of the first of these scientists was Henneberg[10], who proposed the use of an intestinal *Lactobacillus acidophilus*to produce what he called *Acidophilus-Milch,* or reform yogurt. This type of fermented product

finally became a success under the name of yogurt mild in Germany and some other Western European countries in the early 1980s. "Mild yogurt" is made with slightly different cultures to those used for normal yogurt. In this case the *Lactobacillus bulgaricus*is omitted and other lactobacilli are used instead. The probiotic bacteria used in commercial products today are mainly members of the genera *Lactobacillus, Bifidobacterium* and Yeast but other microbes are also used. The effectiveness of probiotics depends on their survival through both the acidic stomach environment and the alkaline conditions of the duodenum, as well as the ability to adhere to the intestinal mucosa of the colon[11]. Research has been carried out to prove the survival ability of probiotic in the GIT. There are evidences that probiotic have survived the gastrointestinal effects and adhere well to the intestinal mucosa[12].Thus, the aim of the present study was to gathered sufficient information on the use of probiotics in most common digestive illnesses like Acute and Chronic Diarrhea. Some volunteers were also studied to quantify the effects of probiotics in healthy consumers.

MATERIAL AND METHODS

Probiotics of three different brands were purchased from the market and isolated in pure cultures, identified and freeze dried. The freeze dried probiotics were designated as laboratory prepared probiotics. The scientists have [13]suggested a minimum viable number of 106 CFU/ml or gram but recommends 108 CFU/g to compensate for reduction through passage through the gut.A typical daily dose should supply about 3 to 5 billion live microorganisms[14].The typical dose of *Saccharomyces boulardii* yeast is 250 mg twice daily[15].These probiotics were filled aseptically in capsules.

Design of clinical study

A blind and placebo control clinical study was conducted to demonstrate the effectiveness of laboratory prepared probiotics. A total 75 patients suffering from acute and chronic diarrhea were studied and 30 healthy volunteers were studied as control. The studies were carried out at different localities of Lahore and Peshawar city. All the healthy subjects were the residents of Girls Hostel-I, Quaid-e-Azam campus Punjab University Lahore city, Pakistan. However, all the patients were studied at the two private Clinics in Peshawar city, Pakistan. The effectiveness of the treatment was assessed by the recovery of the ailment and improvement of the symptoms associated with the illness. Dehydrated skimmed milk was used as placebo. The freeze dried powder and the placebo was given in the form capsules. Freeze dried probiotics and placebo was filled in the capsules by manual hand filing capsule machine (SIVIER MM-34) at the college of

Pharmacy, Punjab University Lahore, Pakistan. Each capsule contain 251 mg *of Saccharomyces boulradii* (2.3 x 10^8 cfu / g), 20 mg of *Bifidumbacteriumbifidus* (2.6 x10^8 cfu / g) and 35 mg of *Lactobacillus acidophilus* (2.1 x 10^8 cfu / g). The study performa-1used during the study can be seen in appendix –A

Inclusion and exclusion criteria for the selection of the healthy volunteers / patients

The healthy volunteers/patients were selected on the basis of few standards. The inclusion criteria means a standard for the including healthy volunteers / patient who are eligible for the clinical study. The exclusion criteria means a standard for excluding healthy volunteers/ patients who were not eligible for the clinical study. The details about these criteria can be seen in Table-1.

Table -1 Inclusion and exclusion criteria for the patients

	Inclusion criteria	Exclusion criteria
Healthy volunteers	No one of the volunteer should be suffering from any digestive illness. All the patients should be adult.	Any subject suffering any digestive illness. Subjects less than 18 years of age.
Acute diarrhea	More than 3 liquid stools in the last 24 hours. Patient age should not be less than 18 years.	a. Chronic diarrhea b. Drug induced diarrhea. c. Bloody stools. d. Crohn's disease
Chronic diarrhea	Patient between age of 18 to onward. Patient with history of giardiasis, shigellosis and diarrhea of unknown origin	Patients less than 18 years of age. Patient suffering from acute diarrhea. Patient suffering from drug induced diarrhea

Demonstration of effects of combine probiotics in healthy volunteers

For the clinical studies thirty healthy volunteers were selected. These volunteers were divided in to two groups, Group A and Group B**.** Group Are presented the active group and Group B represented the placebo control group. Group A was further divided into three sub-groups, A-1,A-2 and A-3.Eachsub -group was consisted of five subjects. The laboratory prepared probiotic was given in the form of capsules. Each capsule contained 35 mg *Lactobacillus acidophilus* (2.1 x 10^8 cfu/ gm) , *Bifidumbacteriumbifidus* 20 mg (2.6 x 10^8cfu/ gm) and 251 mg yeast (2.3 x 10^8 cfu/g) *Saccharomyces boulradii*. Group A-1 had taken one capsule of *Lactobacillus acidophilus* twice a day, Group A-2 has taken 1 capsule of *Bifidumbacteriumbifidus* twice a day and Group-3 had taken 251 mg *Saccharomyces boulradii* twice a day. However, Group B had taken dehydrated skimmed milk as placebo in the form of capsules for one week in the same quantity given to the Group A.

Demonstration of clinical efficacy of *Lactobacillus aciduphilus*in patients with acute diarrhea

For the clinical study twenty one patients were selected who were already being suffering from acute diarrhea. These patients were divided in to two groups, Group A and Group B. Group A represented the active group and Group B represented the placebo controlled group. Group A was consisted of eleven patients. These patients of acute diarrhea were treated laboratory prepared freeze dried *Lactobacillus acidophilus* in the form of capsules for one week. Each capsule contain 35 mg of freeze dried *L. acidophilus* (2.1 x10 ^{8}cfu / gm). The dosage regimen of the Group A was as follows:

1stand 2nd day treatment = one capsule thrice a day.

3rd to 7th day treatment = one capsule daily.

Group B was consisted of ten patients and had taken dehydrated skimmed milk as placebo in the form of capsules for one week in the same quantity given to the Group A. The clinical observations are summarized in the Table-2.

Demonstration of clinical efficacy of *Saccharomyces boulardii*in patients suffering from chronic diarrhea

For demonstration of clinical effectiveness of probiotics against chronic diarrhea twenty patients were selected who were already suffering from chronic diarrhea. These patients were divided in to two equal groups, Group A and Group B. Group A represented the active group and Group B represented the placebo controlled group. Group A was further divided into three sub-groups, A-, A-2 and A-3. Group A-1 was consisted of five patients and already had been diagnosed Giardiasis and they were being treated with[16]tinidazole 300 mg twice daily. After this treatment, their stool was microbiologically tested and the microbial test confirmed the absence of the protozoal cysts in the stool but the diarrhea continued. GroupA-2 was consisted of four patients who had already been diagnosed the shigellosis and these patients were being treated with [16] trimethoprim 80 mg+ sulfamethexazole 400 mg twice daily. After the treatment, the microbiological test shown the absence of microbes from the stool but the diarrhea continued. Group A -3 was consisted of only one patient who was suffering from chronic diarrhea of unknown cause. No therapy was effective to control diarrhea of the patient. All the patients in Group A were treated with laboratory prepared freeze dried *Saccharomyces boulardii*, 251mg (2.3 x 10^{8} cfu/gm) twice a day in the form of capsules up to three weeks. Group B was consisted of ten patients. Group B was further divided into three sub-groups, B-1,B-2,B-3. Group B-1 was

consisted of five patients and already had been diagnosed Giardiasis and they were being treated with[16]tinidazole 300 mg twice daily. After this treatment, their stool was microbiologically tested and the microbial test confirmed the absence of the protozoal cysts in the stool but the diarrhea continued. GroupB-2 was consisted of three patients who had already been diagnosed shigellosis and these patients were being treated with trimethoprim 80 mg + sulfamethexazole 400mg twice daily. After the treatment, the microbiological test shown the absence of microbes from the stool but the diarrhea continued. Group B-3 was consisted of two patients and who were suffering from chronic diarrhea of unknown cause. No therapy was effective to control diarrhea of these patients. All the patients in the Group B had taken dehydrated skimmed milk as placebo in the same quantity as received by Group-A. The clinical observations are summarized in Table -3.

RESULTS AND DISCUSSION

The responses of Group A of healthy volunteers after taking Probiotics were: No volunteer had noted any change in the appetite (increase or decrease), thirteen volunteers reported that during the period of therapy with the probiotics constipation or diarrhea did not develop, only two of the volunteers reported constipation during the use of probiotics which may be due to other factors including the diet. Three of the control volunteers who had be given skimmed milk as placebo reported softness of stool during the first and second day while taking placebo .Two control volunteers reported increased frequency of stool. The placebo control volunteers also did not noted any change in appetite (increase or decrease). The clinical study conducted was blind and placebo control. The design of the clinical study was blind because it was predicted that it would be almost impossible to persuade healthy volunteers / patients to take live microorganisms for control of the disease they were suffering from. Placebo was used for psychological effects or as a control to differentiate the effectiveness of the probiotics to the active group. The patients suffering from acute diarrhea showed an improvement of the diarrheal episodes from 3-4 liquid stools per day to one solid stool per day immediately when treated with laboratory prepared freeze dried *Lactobacillus acidophilus*. The patients with acute diarrhea have 3 to 4 liquid stool on average in last 24 hours with the use of *Lactobacillus acidophilus*(laboratory prepared probiotic)the frequency of stool reduced to 1 to 2 semisolid stools per day after two days of treatment and with-in 7 days of treatment the frequency of stool become one with solid consistency. Similarly, the nausea sensation felt of diarrheal episodes had improved with -in two days of treatment and finally no such sensation was felt after 7 days of treatment. When patients suffering from chronic diarrhea were treated with laboratory prepared freeze dried

Saccharomyces boulardii their stool frequency was reduced from 5-6 liquid stools per day to 1-2 semisolid stools per day. With continuous use the chronic diarrhea was controlled.

Table -2: Observations for the demonstration of clinical efficacy of *Lactobacillusacidophilus* in patients suffering from acute diarrhea

	Before Treatment		**After 2 Days of Treatment**		**After 7 Days of Treatment**	
	Group A	**Group B**	**Group A**	**Group B**	**Group A**	**Group B**
No of patients	11	10	11	10	11	**10**
Stool frequency/ day	3-4	3-4	1-2	3-4	1	**3-4**
Stool consistency	Liquid	Liquid	Semisolid	Liquid	Solid	Liquid
Nausea	Felt	Frequent	Improved	Frequent	Not felt	Frequent

Table -3: Observations for demonstration of clinical efficacy of *Saccharomyces boulardii*in patients suffering from chronic diarrhea

Symptoms	**Group A**		**Group B**	
	No of patients Before treatment	**10 After treatment**	**No of patients Before treatment**	**10 After treatment**
No of stools / day	5-6	1-3	5-6	5-6
Stool consistency	Liquid	Semisolid	Liquid	Liquid

CONCLUSION

From the results of clinical study it can be concluded that probiotics can work as an alternative and effective treatment for the control of acute diarrhea and can improve symptoms of chronic diarrhea. With continuous use it is possible to get rid of chronic diarrhea completely. In addition, continuous use of probiotics is an effective way of development of a healthy gut and a development of immunity against intestinal infections. More ever, the health-promoting bacteria able to exert a positive impact on the intestinal microflora. Thus, it is proposed that the beneficial bacteria that inhabit the human body may promote good health by outweighing the negative activities of harmful commensal and invading microbes. It is also evident from the study that probiotic micro-organisms can survive the extreme pH conditions of stomach and small intestine to exert their beneficial effects.

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Appendix -A

Study Performa-1			
Aim of the study : Demonstration of clinical efficacy of probiotics in acute and chronic diarrhea			
Type of study: Blind and Placebo control study.			
Inclusion criteria			
Exclusion criteria			
Dosage regimen			
Time interval for the dose	Two times a day		Three times a day
Duration of treatment	Days	Weeks	Month

Patient Performa-2			
Patient name :			
Age:			
Gender :			
Nature of illness			
	Acute diarrhea	Chronic diarrhea	
Duration of illness	Days	Weeks	Months
Physician name:			
Date of starting treatment :			
Date of ending treatment:			
Type of probiotic microorganism given in the present studies:			
Lactobacillus acidophilus (2.1 x10^8 cfu / g, approximately) *Bifidumbacteriumbifidus*(2.6 x 10^8cfu / g, approximately) *Saccharomyces boulradii* (2.3 x 10^8cfu / g, approximately)			
Dosage regimen:			
No of capsules per day			
Time interval for the dose	Two times a day		Three times a day
Duration of treatment	Days	Weeks	Month
Signs and Symptoms before treatment: a. ---------- b. ----------			

c. ----------
Sign and symptoms after treatment: a. ---------- b. ---------- c. ----------
Result/ Conclusion:
Improvement a. Complete cure b. Partial improvement
Relapse:
Treatment after relapse :
Improvement after relapse a. Complete cure b. Partial improvement