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IMMUNOPROPHYLACTIC EFFECT OF PROBIOTIC YOGHURT FEEDING ON *SCHISTOSOMA MANSONI*-INFECTED MICE

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This work studied the immunmoprophylactic effect of probiotic yoghurt administration to mice before and after infection with *Schistosoma mansoni*. Female Swiss albino mice were divided into four groups (G1, G2, G3 and G4), G1 and G3 were fed on the basal diet, G2 and G4 were fed on the basal diet and probiotic yoghurt. After feeding, mice of groups G 3 and G 4 were infected with 100 cercariae of *Schistosoma mansoni*. Their livers and spleens were removed and their weights were recorded. Plasma were separated and used for the determination of IgM level, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γGT). The results obtained showed that the mean liver and spleen weights were significantly increased in infected mice group (G 3) as compared with the control (G 1). Supplementation of probiotic yoghurt (G 2) decreased the spleen and liver weights to be nearest the control. In addition, probiotic yoghurt was found to display an immunomodulatory effect by stimulating IgM response against soluble worm antigen preparation (SWAP) as compared to the normal control. Also, there was an increase in the IgM level in infected mice fed on probiotic yoghurt as compared to the infected mice not fed on probiotic yoghurt. In addition, the activity of AST, LDH and γGT was significantly increased in the infected group compared with the control. The addition of probiotic yoghurt to a diet in the infected group led to a decrease in the activity of AST, LDH and γGT to be nearest to the control. Hence, we concluded that feeding with probiotic yoghurt resulted in a significant stimulation for IgM response against SWAP before and after infection with *Schistosoma mansoni*. In addition, the liver and spleen weight were decreased in probiotic yoghurt-infected mice as compared to the non-yoghurt infected mice. Also, the supplementation of yoghurt led to a decrease in the activity of AST, LDH and γGT in the infected mice to be nearest to the control.

INTRODUCTION

Functional foods that are claimed to enhance the immune system have mainly been fortified with vitamins, whey proteins or contain probiotic cultures. Some probiotics have the ability to boost the immune system, and the antioxidant vitamins A, C and E can increase the resistance of the body to infection. Many of these products have also an effect on heart or gut health [Spanhaak *et al.*, 1998, Fox & Flynn, 1992; IFIC, 1999].

Dairy products dominate the area of functional foods for gut health. Common gut health products include fermented milk and yogurt drinks. The most common gut health ingredients include probiotics, prebiotics and synbiotics.

Probiotics are beneficial bacteria that help to maintain the balance of beneficial and harmful bacteria in the gut whereas prebiotics are a natural food for probiotic bacteria thus supporting their growth. A synbiotic product contains both a probiotic and a prebiotic ingredient. Beneficial bacteria (probiotic cultures), can actually strengthen the immune system's resistance to infection and improve ther overall gastrointestinal health [Agerholm-Larsen *et al.*, 2000].

The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. The immune system is constantly active, acting to discriminate "non-self" from "self". The immune system has two functional divisions: the innate and the acquired. Both components involve various blood-borne factors (complement, antibodies, cytokines) and cells. Nutrient status is an important factor contributing to immune competence: malnutrition impairs the immune system, suppressing the immune functions that are fundamental to host protection. There is increasing evidence that probiotic bacteria improve the immune function of a host. The effect of enhancing the immune function on host resistance to infection in healthy individuals is not, however, clear [Calder & Kew, 2002].

Probiotics are friendly bacteria found in the mouth and intestines of healthy individuals and in the female vagina. These microorganisms help to defend the body against invading bacteria and yeasts. Probiotic bacteria contribute to gastrointestinal health by providing a synergistic environment and producing health-promoting substances including some vitamins [Agerholm-Larsen *et al.*, 2000]. They can regulate bowel movements and halt diarrhea while at the same time enhancing the immune system. Schistosomiasis as

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a disease is estimated in countries around the world, from temperate Africa amount to hundreds of millions of dollars annually [WHO, 1995].

In Egypt, the prevalence rate of Schistosomiasis is very high and about 20 millions of its population are affected [Madwar *et al.*, 1983]. It is estimated that about 20% of those heavily infected individuals with *Schistosoma mansoni* may have splenic enlargement and a firm or shrunken liver [Mousa, 1975]. Schistosomiasis is a chronic infection which involves extensive interactions between various life cycle stages of the parasites and the immunological system of the host.

Several immunoregulatory mechanisms, both humoral and cell-mediated, may be involved in the pathogenesis of clinical Schistosomiasis [Borose, 1989]. One of the main concepts that has emerged in recent years is that the potential control of Schistosomiasis relays on multiple and integrated strategies, among which the modern tool of immunointervention (both for prophylactic and possibly therapeutic purposes) will play a significant, if not the major, role [Capron, 1998].

Schistosoma mansoni is dangerous parasite. In its life cycle, it is able to infect humans, where its main settling site is liver. Therefore, issues described in the paper are of special importance to human health.

The aim of the present study was to introduce yoghurt supplemented with *Lactobacillus casei*, plantarum, reutrie and acidophilus bacteria into mice diets and to monitor changes in the immunological and biochemical parameters in order to prove the protective effect of the bacteria examined on the course of infection with *Schistosoma mansoni*.

MATERIALS AND METHODS

Experiment. Female Swiss albino mice of an initial body weight of 16-19±2 g were randomly divided into four test groups (n=5): the first group (G 1) was fed on the basal diet without infection; the second group (G 2) was fed on basal diet + probiotic yoghurt (5 g/ mice) without infection; the third group (G 3) was fed on basal diet with infection; the fourth group (G 4) was fed on basal diet + probiotic yoghurt (5 g/mice) with infection.

Within each feeding trail, the composition of the basal diet in each group was identical in its protein, crude fat, sucrose and crude fiber. The composition of minerals [Williams & Briggs, 1963] and vitamin mixture [Muller, 1964] were incorporated in the diets at respective levels of 3.50 and 2.0%, while corn starch was added to make the diet weight of 100 g. The composition of basal diets used in this study was as follows: milk protein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%), and starch (64%).

The feeding period was three weeks for adaptation and it continued two weeks after infection. At the end of the feeding and infection, each mouse was anesthetised and blood was collected from orbital plexus on Na2-EDTA (1 mg/1 mL blood). The mice were killed and livers and spleens were excised and weighed.

Bacterial strains. Strains of *Lactobacillus casei* B-444, *Lactobacillus plantarum* B-531, and *Lactobacillus reutrie* B-14141 were provided by Northern Regional Research Laboratory, Illinois, USA (NRRL). *Lactobacillus acidophilus* was from Chr. Hansen's Lab., Denmark.

Preparation of probiotic yoghurt. Skim milk powder, (Valio, Finland) was dissolved and standardised to achieve the desired contents, then pasteurised for 30 min at 85°C, and then cooled to 40°C. *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus reutrie* strains were inoculated individually and incubated for 4–6 h at 37°C and cooled and stored at refrigeration temperatures (5°C). Yoghurts containing probiotic strains (10^6) of *L. casei*, *L. acidophilus*, *L. plantarum* and *L. reutrie* strains were mixed together for feeding.

Parasite and infection. Cercariae of *Schistosoma mansoni* were shed by illuminating infected *Biomphalaria alexandrina* snails obtained from the biological supply unit, Theodor Bilharze Institute, Giza, Egypt and used to infect mice. Each mouse received 100 cercariae subcutaneously [Smithers & Terry, 1965].

Enzyme activity determination. Enzyme activity of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γGT) was determined by kits of BOEHRINGER, Germany.

Detection of IgM response against soluble worm antigen preparation (SWAP). The assay was performed according to Hillyer and Gomez [1979]. The plate was coated with SWAP (100 μL/well of 50 μg/mL of coating buffer) and incubated overnight at room temperature, then washed three times using Tween 20 (0.05%) in phosphate buffer saline (PBS). Plates were blocked for sites free of antigen using 200 μL/well of Tween 20 (0.05%), bovine serum albumin (BSA) (1%) in PBS to fill the wells, then left for 1 h at room temperature and washed three times using working buffer. A volume of 100 μL/well of diluted normal infected and treated infected mice sera at the dilution of 1:100 in blocking buffer was added. The plates were incubated at 37°C in a shaking water bath for 2 h, then washed three times using washing buffer. Anti-mouse IgM peroxidase conjugate was added 100 μL/well at the dilution of 1:10000 in blocking buffer and incubated for 1 h at 37°C. Orthophenylene diamine dihydrochloride (*O*PD) was diluted in substrate buffer. The change in optical density was read at a wavelength of 490 nm using an Automatic Titertek Multiskan Reader.

Statistical analysis. Statistical analysis of variance (t-test) within groups and between groups was conducted as described by Miller and Miller [1992].

RESULTS AND DISCUSSION

The immune system is an intricate network of specialised tissues, organs, cells, and chemicals. The lymph nodes, spleen, bone marrow, thymus gland, and tonsils play a role the immune system as well as lymphocytes (specialized white blood cells), antibodies, and interferon. Two types of immunity protect the body: innate and adaptive.

The innate immunity is present at birth and provides the first barrier against microorganisms. The skin, mucus secretions, and the acidity of the stomach are examples of the innate immunity that act as barriers to keep unwanted germs away from more vulnerable tissues.

Infection is the result of invasion of the body by microorganisms, including bacteria, viruses, or fungi. Not all microorganisms cause infections in the body, and exposure to a disease causing microorganism does not always result in symptoms. The immune system plays an important role in determining the body's ability to fight the infection.

Body weight, organ weight and macroscopic examination of liver and spleen

The results obtained showed that the body weight gain was significantly ($p \leq 0.05$) increased in experimental group fed with probiotic yoghurt diet as compared to the control group (Table 1). Table 1 shows that the mean liver weight, spleen weight, ratio (organ/body weight) was significantly increased ($p \leq 0.05$) in the experimentally-infected group of mice fed with probiotic yoghurt diet as compared to the control group. The supplementation of probiotic yoghurt decreased the liver and spleen weight to be nearest to the control. Fiore *et al.* [1996] reported that *Schistosoma mansoni* infection reduced the body weight of infected mice after 8 weeks, while in the present study up to there was reduction in the body weight of infected mice after two weeks.

Macroscopic examination showed no change in liver appearance in both mice fed with probiotic yoghurt and basal diet I.

Macroscopic examination carried out two weeks following the subcutaneous infection with 100 live *Schistosoma mansoni* cercariae showed a few gray lesions in control--infected mice. Bloch [1980] reported that during *Schistosoma mansoni* infection, punctuate grey 0.5 mm lesions, increase gradually in number and size and the liver darkens in colour and increases in size.

The macroscopic appearance of spleen in mice fed with probiotic yoghurt and basal diet was undistinguishable.

The weights of spleen taken from infected mice (0.15 ± 0.02 g) were enlarged and increased significantly, reaching 3.26 times of the normal value (Table 1). In probiotic yoghurt-fed infected group (0.046 ± 0.029 g) it did not differ significantly from that of the probiotic yoghurt-fed non-infected group (0.049 ± 0.02 g).

The macroscopic appearance of spleen in infected mice was slightly enlarged and become red in appearance. In contrast, the macroscopic appearance of spleen in probiotic yoghurt-fed mice before and after infection was undistinguishable.

Enzyme activities of transaminases (AST& ALT), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γGT)

The hepatic transaminase aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are sensitive indicators of hepatocellular injury [Reichling & Kaplan, 1988].

Table 2 demonstrates the effect of dietary yoghurt on transaminases (AST and ALT), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γGT) activities in plasma. The data obtained showed that *Schistosoma mansoni*-infected mice did not have any marked effects on ALT activity however; AST, LDH and γGT activity was significantly increased in the infected group compared to the control ($p \leq 0.05$).

TABLE 2. Effect of dietary probiotic yoghurt on transaminase (AST and ALT), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γGT) activities in plasma.

Experimental groups	ALT	AST	LDH	γGT
	U/L			
G 1	103±3.1	122± 4.5	937±12.1	17.77±0.9
G 2	105±3.5	123± 4.4	935±13.1	16.91±0.8
G 3	104±2.9	225± 5.1	2340±20.5	50.11±1.11
G 4	103±4.7	125.8*±4.5	1164*±15.1	22.22*±0.93

* – p-values are based on t-test at a significance level of 0.05; S.E. – standard error

Ahmed and Gad [1995] reported that infection with *Schistosoma mansoni* caused the following changes in mice livers, which are remarkable increase in the activities of liver pyruvate kinase and phosphoructokinase from the 4th week of infection. These results led to the conclusion that glycolysis is largely stimulated in the liver of infected mice at the expense of other metabolic pathways of glucose utilisation.

The revealed data demonstrated that the addition of probiotic yoghurt to diet in the infected group led to a decrease in the activity of AST, LDH and γGT to be nearest to the control ($p \leq 0.05$).

Effect of probiotic yoghurt feeding on IgM level of *Schistosoma mansoni*-infected mice

The humoral immune response induced by probiotic yoghurt feeding was determined by the detection of IgM

TABLE 1. Immunmoprophylactic effect of probiotic yoghurt feeding on spleen and liver weights before and after infection of mice with *Schistosoma mansoni*.

Experimental groups		Body weight (g)	Liver weight (g)	Ratio (a)	Spleen weight (g)	Ratio(a)
G 1	Mean	16.0	0.643	0.040	0.036	0.002
	S.E.	1.20	0.10		0.01	
G 2	Mean	19.0	0.686	0.036	0.049	0.02
	S.E.	1.10	0.10		0.02	
G 3	Mean	15.50	0.821	0.053*	0.150	0.005*
	S.E.	1.50	0.12		0.02	
G 4	Mean	17.50	0.621	0.035	0.046	0.004
	S.E.	1.20	0.13		0.03	

A – organ/body weight (g/g); * – p-values are based on t-test at a significance level of 0.05; S.E. – standard error

level against SWAP before and after *Schistosoma mansoni* infection (Table 3).

TABLE 3. Effect of probiotic yoghurt on IgM levels before and after infection of mcie with *Schistosoma mansoni* (results expressed as absorbance readings at 490 nm).

Experimental groups	Min	Max	Mean	S.E.
G 1	0.050	0.08	0.066	0.01
G 2	0.279	0.286	0.336	0.005
G 3	0.304	0.367	0.283	0.003
G 4	0.308	0.621	0.388	0.12

The level of IgM (0.336±0.02) was significantly increased (p=0.005) in probiotic yoghurt-feeding mice as compared to the control (0.066±0.01). In addition, the level of IgM increased in the group fed with probiotic yoghurt-infected with *Schistosoma mansoni* (0.387±0.12) as compared to control – infected with *Schistosoma mansoni* (0.283±0.003).

The present study demonstrated that probiotic yoghurt has an immunomodulatory effect by stimulating IgM response against SWAP in sera of fed mice as compared to non-fed ones. In addition, our results proved the immunomodulatory effect of probiotic yoghurt by the increasing IgM level in the group of mice fed with probiotic yoghurt-infected with *Schistosoma mansoni* compared to the groups fed with basal diet with infection or as compared to the group fed with probiotic yoghurt without infection. Our results are in agreement with those of Hanson and Yolken [1999], who revealed that probiotic bacteria have been shown to reinforce the different lines of gut defense; immune exclusion, immune elimination, and immune regulation. They have been shown to enhance humoral immune responses and, consequently, to promote the intestine's immunological barrier. Probiotics have also been shown to stimulate non-specific host resistance to microbial pathogens and thereby aid in their immune elimination. In addition, our results are convenient with the findings reported by Bloksma *et al.* [1979], that lactobacilli are known for their health-promoting effects such as non-specific enhancement of the immune system, protection against intestinal infection, decreasing cholesterol levels in serum, and anti-carcinogenic activity.

CONCLUSIONS

Probiotic yoghurt displays an immunmoprophylactic effect by stimulating plasma immunoglobulin response, which improved the liver and spleen weight to be nearest to the control. In addition, the activities of aspartate transaminase (AST), lactate dehydrogenase (LDH) and γ-glutamyl transferase (γGT) were significantly increased in the infected group compared to the control.

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