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Effectiveness of Probiotic Labneh Supplemented with Garlic or Onion Oil Against *Schistosoma mansoni* in Infected Mice

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Abstract: The aim of the present study is to investigate the preparation and biological evaluation of a probiotic labneh synergistic with garlic and onion oil against *Schistosoma mansoni* in infected mice. The results pointed out that, probiotic labneh containing garlic and onion oil have demonstrated an anti-schistosomal activity against *Schistosoma mansoni*-infected mice. The levels of protection reduction ratio in total worm couple, male and female ratio were 66.67, 57.14 and 50.0% in mice infected with *Schistosoma mansoni* fed on probiotic labneh with garlic oil and were 50, 57.14 and 50% with onion oil, respectively. The mean percentage of reduction in total ova count in liver were 70.0 and 56.44% in mice infected with *S. mansoni* and fed on probiotic labneh containing garlic and onion oil, respectively. The mean values of immature, mature and dead oogram in mice infected with *S. mansoni* and fed on probiotic labneh containing garlic oil were 44.0±1.47, 43.0±3.5 and 7.0±2.5, respectively and 47.0±2.82, 46.0±0.77 and 5.0±0.57 in mice infected with *S. mansoni* and fed on probiotic labneh containing onion oil respectively. Data, also, showed that probiotic labneh containing garlic and onion oil increased the dead oogram in mice infected with *S. mansoni*. Data can be concluded that probiotic labneh containing garlic and onion oil may be play a great role as a protective food to control parasites.

Key words: Probiotic, Labneh, garlic oil, onion oil, *Schistosoma mansoni*, infected mice

INTRODUCTION

Schistosomiasis is a serious health problem since it affects about 200 millions of the world population in developing countries, while 400-600 millions are exposed to the risk of infection. The importance of the disease lies in the fact that it affects not only the overall health status and fitness of the people, but also the human productivity and national economy. There is thus a need to apply efficient methods for transmission control in conjunction with chemotherapy and vaccine development. In recent time the concept is providing functional foods containing healthful components rather than removing potentially harmful ones. Functional foods, designer foods, pharma-foods and nutraceuticals are synonyms for foods with ingredients that can prevent and treat diseases (WHO, 1995; Madwar *et al.*, 1983, 1989). Flat worms belong to the genus *Schistosoma*; includes species which are parasitic in humans and domestic animals. At least more than 200 million people suffer from schistosomiasis (*Bilharzia*) in the tropics and sub-tropics. Infection occurs through direct penetration of the skin by the water-dwelling infective stage; thereafter the parasite (which achieve 1-3 cm in length) are blood-dwelling either in the veins around the bladder or the intestine, depending on the species involved (Atkinson *et al.*, 1999).

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A functional food is similar in appearance to, or may be, a conventional food, is consumed as part of a usual diet and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions (Goldberg, 1994).

Labneh is a type of dairy products manufactured from milk and fermentation was carried out using lactic acid bacteria. A probiotic is a live microbial feed supplement which beneficially effects on the host animal by improving its intestinal microbial balance. Hence, it may be considered as a functional food with the special property of containing live and beneficial microorganisms (Fuller, 1992; McIntosh, 1996).

Garlic (*Allium sativum*) and onion (*Allium cepa*) are used all over the world for different diseases (Ibn-cenna, 980-1937 A.P.). More than 4000 publications have provided evidence for the efficacy of these herbs in the prevention and treatment of a variety of diseases and for validating its traditional uses. It has been shown that garlic has different applications as antimicrobial (Azad Chowdhury *et al.*, 1991; Yoshida *et al.*, 1998), antitumor (Karasaki *et al.*, 2001; Sundaram and Milner, 1996), antithrombotic, hypolipidaemic, antiarthritic and hypoglycemic agent (Duraka *et al.*, 2002; Kumar *et al.*, 2003).

The therapeutic use of probiotics has been considered as successful in the cases of lactose intolerance, certain diarrheal diseases, Inflammatory Bowel Disease (IBD) and atopic eczema (Fuller, 1992; Mercenier *et al.*, 2002). Positive indications have been obtained (and which still need to be reinforced) for Irritable Bowel Syndrome (IBS), colon cancer, travelers diarrhea and *H. pylori* infection. In contrast, insufficient proof has been provided for some effects on viral infections or blood cholesterol reduction. They are used for modulation of the immune system improving resistance to chemicals, inflammation and other factors by binding and/or degradation of potential carcinogens (improved intestinal metabolic activity). They also used for alteration of the metabolic activities of the intestinal microflora (production of antitumorogenic and for antimutagenic compounds) and for improvement (quantitatively/qualitatively) of the intestinal microflora, reducing the putative producers of carcinogens and cancer promoters (improvement of the intestinal microecology). This can be achieved by more bile-acid degrading bacteria; less bacteria producing the enzymes azoreductase, nitroreductase, betaglucuronidase, beta-glucosidase etc. (Mercenier *et al.*, 2002).

The aim of the present study is to evaluate the effectiveness of probiotic labneh containing garlic or onion oil against *Schistosoma mansoni* in infected mice.

MATERIALS AND METHODS

Milk Protein Concentrate

ALAPRO™ 4700 milk protein concentrate powder was purchased from NZMP Co., New Zealand. The milk protein concentrate composition was 4.4% moisture, 69.8% total protein, 14% fat, 7.2% ash and 17.20% lactose.

Garlic and Onion Oils Preparation

Garlic and onion oils were extracted and purified from the Egyptian crops using local pressing and squeezer, Cairo, Egypt.

Traditional and Probiotic Cultures

Starter cultures of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and DVS-ABT2 (containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) were purchased from Chr. Hansen's Lab., Copenhagen, Denmark.

Preparation of Traditional and Probiotic Labneh

Milk protein concentrate powder (ALAPRO™, NZMP Co., New Zealand) was reconstituted and standardized to achieve 16% total solids contents and then heated at 85°C for 30 min, cooled to 40°C and divided into two portions:

- The first portion (P₁) was inoculated with DVS-ABT2 starter (containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) and supplemented with 3% garlic oil (manufactured in local pressing and mangle).
- The second portion (P₂) was inoculated with DVS-ABT2 starter (containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) and supplemented with 3% onion oil (manufactured in local pressing and mangle).

All portions were incubated for 4-8 h at 42°C. After coagulation, the curd was pH tested and stored at 4-6°C±2.

Diets

The compositions of basal diets used in this study are milk protein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), mineral mixtures (4%), dietary fiber (4%) and starch (64%).

Animals

Thirty female Swiss albino mice (18-25 g) were randomly divided into three-test groups (each containing 10 mice). Experimental animals were placed in cages and given the basal diets only or basal diet supplemented with probiotic labneh containing garlic and onion oil for 21 before infection and then, 45 days after infection:

- The first group (G₁) animals were fed on the basal diet plus probiotic labneh containing 1% garlic oil (5 g/mouse/day).
- The second group (G₂) animals were fed on the basal diet plus probiotic labneh containing 1% onion oil (5 g/mouse/day).
- The third group (G₃) animals served as control and were fed on basal diet.

Infection and Experimental Groups

After twenty one days post feeding mice were infected subcutaneously (S.C.) with 100 *Schistosoma mansoni* cercariae per each mouse.

Assessment of Worms Burden

Perfusion and recovery of adult worms were performed at 6 weeks post infection through hepatic portal vein by the perfusion method (Smithers and Terry, 1965). The total tissue worms in liver and intestine were determined. Protection was assessed as the percentage reduction in worm counts in liver and intestine according to the formula:

$$P = \frac{C - T}{C} \times 100$$

Where:

P = Percentage reduction of worms

C = Mean worm burdens in control infected animals

T = Mean worm burdens in pre-treated infected animals

The percentage reduction in ova counts in liver and intestine can were also, calculated according to the previous equation.

Mean and standard Error of the obtained data from each different experimental group were calculated and analyzed according to the method described by Miller and Miller (1992).

Methods of Analysis

Chemical composition and sensory evaluation for the appearance, color, flavor and overall properties of synbiotic fermented milk were adopted from NASA (1999) and analyzed according to official method (AOAC, 1990). Sensory evaluation were carried out using twenty panelists with three categories : (+++) very good (++) good, (+) accepted, (-) unaccepted.

RESULTS AND DISCUSSION

The food industry is the largest industry in the world aiming to increase in the development of foods that promote health. It includes foods such as natural functional foods (Honey, herbs, fermented milk and cereal, cranberry juice, green tea etc.), foods and ingredients for specified health use, formulas (infant and elderly), medical foods, nutraceuticals and drug foods. Within this continuum between food and drug, there are unlimited niches for the development of food systems that promote optimal health and general well-being. Probiotic and herb have a therapeutic effect that are responsible for enhancing human health and nutrition (Fuller, 1992).

Chemical composition and nutritional values of probiotic labneh (g/100 g) are shown in Table 1.

Sensory evaluation of probiotic labneh containing garlic and onion oil are presented in Table 2. The results indicated that labneh with garlic or onion oil rated lower in the sensory evaluation than plain labneh but that they were still good and acceptable in all categories.

The effect of probiotic labneh containing garlic and onion oil have demonstrated an anti-schistosomal activity against *Schistosoma mansoni*-infected mice. The obtained results showed that probiotic labneh containing garlic and onion oil have a protective effect against worm burden of *S. mansoni* in infected mice compared with control (Table 3). Moreover, data

Table 1: Chemical composition and notional values of probiotic labneh (g 100 g)

Ingredient	Carbohydrate	Fat	Protein	Ash	Total solids
%	6.16	4.23	4.51	0.95	15.95
Calories	24.64	38.07	18.04	-	80.75

Table 2: Sensory evaluation properties of synbiotic fermented milk

Sensory evaluation	Probiotic labneh containing garlic oil	Probiotic labneh containing onion oil	Plain probiotic labneh
Appearance	++	++	+++
Color	++	++	+++
Odor	+	+	+++
Flavor	+	+	+++
Overall	++	++	+++

+++ : Very good, ++: Good, +: Accepted, -: Un-accepted

Table 3: Effect of probiotic labneh containing garlic and onion oil on worm burden of *S. mansoni* in infected mice

Diets	Mean±SD		
	Worm Couple	Male	Female
Basal diet	5.0±0.70	7.0±1.2	2.0±0.40
Probiotic labneh with garlic oil	3.0±0.70	3.0±2.1	1.0±0.80
Probiotic labneh with onion oil	2.0±0.40	3.0±0.5	1.0±0.80

showed that the levels of protection against schistosomiasis were 66.67, 57.14 and 50.0% reduction in total worm couple, male and female in mice infected with *S. mansoni* fed on probiotic labneh containing garlic oil. Also, the levels of protection against schistosomiasis in mice infected with *S. mansoni* fed on probiotic labneh containing onion oil were 50, 57.14 and 50% reduction in total worm couple, male and female, respectively (Table 4).

On the other hand, probiotic labneh containing garlic and onion oil reduced ova count in liver and intestine of *Schistosoma mansoni* infected mice compared with infected mice fed on basal diet (control). In this respect probiotic labneh containing garlic oil are more efficient than probiotic labneh containing onion oil (Table 5).

The results exhibit that mean values of total ova count in intestine of infected mice fed on probiotic labneh containing garlic and onion were 613.40±2.75 and 626.87±1.61, respectively, compared with ova count of infected mice fed on basal diet (2723.0±1.37). However, the corresponding values of total ova count in liver of were 625.70±1.59 and 947.2±0.50 for garlic and onion oil, respectively, compared with ova count of infected mice fed on basal diet (2174.6±3.45). These values result in reductions in total ova count in liver and intestine ranging between 56.44-76.98% and 76.98-77.48 in infected mice fed on probiotic labneh containing onion and garlic oil (Table 6). Moreover, garlic oil was more effective in the liver, but the reduction was almost similar for both oil in the intestinal total ova count.

The mean percentage of reduction in total ova count in liver were 70.0 and 56.44% in mice infected with *S. mansoni* and fed on probiotic labneh containing garlic and onion, respectively. The mean percentage of reduction in total ova count in intestine of mice infected with *S. mansoni* and fed on probiotic labneh containing garlic and onion were 77.48 and 76.98%, respectively (Table 6). The

Table 4: Percentage of worm reduction of probiotic labneh containing garlic and onion oil of *S. mansoni* infected mice

Diets	Reduction (%)		
	Worm Couple	Male	Female
Probiotic labneh with garlic oil	66.67	57.14	50.0
Probiotic labneh with onion oil	50.00	57.14	50.0

Table 5: Effect of probiotic labneh containing garlic and onion oil on ova count in liver and Intestine of *S. mansoni* infected mice

Diets	Mean±SD	
	Ova count in liver	Ova count in intestine
Basal diet	2174.60±3.45	2723.00±1.37
Probiotic labneh with garlic oil	625.70±1.59	613.40±2.75
Probiotic labneh with onion oil	947.20±0.50	626.87±1.61

Table 6: Percentage of ova reduction of probiotic labneh containing garlic and onion oil in liver and Intestine of *S. mansoni* infected mice

Diets	liver	Intestine
Probiotic labneh with garlic oil	70.00	77.48
Probiotic labneh with onion oil	56.44	76.98

Table 7: Effect of probiotic labneh containing garlic and onion oil on number of immature, mature and dead Oogram of *S. mansoni*-infected mice

Diets	Mean±SD		
	Immature	Mature	Dead
Basal diet	39.0±1.89	53.0±2.03	5.0±1.89
Probiotic labneh with garlic oil	44.0±1.47	43.0±3.50	10.0±2.50
Probiotic labneh with onion oil	47.0±2.82	46.0±0.77	6.0±1.57

mean values of immature, mature and dead oogram in mice infected with *S. mansoni* and fed on probiotic labneh containing garlic oil were 44.0 ± 1.47 , 43.0 ± 3.5 and 10.0 ± 2.5 , respectively and 47.0 ± 2.82 , 46.0 ± 0.77 and 6.0 ± 1.57 in mice infected with *S. mansoni* and fed on probiotic labneh containing garlic oil, respectively compared with mice fed on basal diet (39 ± 1.89 , 53.0 ± 2.03 and 5 ± 1.89) (Table 7). Probiotic labneh containing garlic oil slightly have more fetal effect on mean values of dead oogram than onion oil in mice infected with *S. mansoni*.

The obtained results showed that probiotic labneh containing garlic and onion oil have been demonstrated an anti-schistosomal activity against *Schistosoma mansoni* infected mice. In this, study, probiotic strains of lactic acid bacteria such as *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were used to prepare the probiotic labneh. Probiotic strains have a beneficially affects in human health and used to correct the traditional nutrient deficiencies by improving its intestinal balance (Fuller, 1992). Rey *et al.* (1983) used milk with oltipraz in the urinary schistosomiasis patients. They used a single dose of 35 mg kg^{-1} with a drug used to treat schistosomiasis (oltipraz), which was given under surveillance together with either whole milk or herrings in oil. The tolerance of the product was very good as only 3% of the patients reported vomiting and 3% paresthesias of the fingers. Sixty six percent (66%) of the patients examined on day 30 and/or on day 90 were egg-negative and egg excretion was reduced by at least 90% in 22 other subjects, bringing the percentage of good results to 74%. On day 90, the mean egg excretion was reduced by 82.5% in the overall population and by 80.9% in the 5 to 14 year-old.

The obtained data are in agreement with data previously reported from large number of epidemiological studies which assessed the influence of milk intake on infectious diseases developed it showed that the major milk proteins, casein, whey proteins and membrane structures, may all exert stimulative effect on the immune system and preventive effect of many of diseases such as cancer and infectious diseases. Whey proteins were found to be protective relative to other protein sources (McIntosh, 1993), this being associated with an increase in the intracellular level of glutathione (GSH), where whey is a prime source of precursors. When liver glutathione levels rise, the liver is able to more effectively detoxification. Un-denatured whey protein optimizes serum and liver glutathione levels (McIntosh *et al.*, 1995).

Also, our result are in agreement with finding reported with Maghraby *et al.* (2005) who investigated the anti-schistosomal activity of colostral and mature camel milk on *Schistosoma mansoni* infected mice. They found that colostral and mature milk of camel have an immunoprotective response against *Schistosoma mansoni*. The study suggested that camel milk can be used with antischistosomal drugs in Schistosomiasis patients. Finding this study are in agreement with the finding of Ghanem *et al.* (2005) who studied the immunoprophylactic effect of yoghurt-containing probiotic feeding before and after infection with *Schistosoma mansoni*. They reported that supplementation of yoghurt containing probiotic decreased the enlarged spleen and liver weights, almost to the control level to be nearest the control. Yoghurt-containing probiotic has immunomodulatory effect and anti-schistosomal activity against *Schistosoma mansoni*. Another study have demonstrated that parasite engulfment and destruction by macrophages is significantly enhanced in *Balb/c* mice treated with either garlic extract or its immunomodulator fraction (Ghazanfari *et al.*, 2005).

Garlic enhances protective immunity against Leishmaniasis is an infectious disease infection by various mechanisms. Garlic has been shown to activate nitric oxide synthase (NOS), the enzyme that produces NO, in platelet and placental villous tissue (Das *et al.*, 1996). On the other hand, activated macrophages produce NO as an important effector molecule and it has been confirmed that control of *Leishmania* infection in the murine model is NO-dependent. Macrophages from resistant strains of mice expressed significantly higher levels of NOS and produced larger amounts of NO compared to the susceptible strains when activated *in vivo* (Mcorley *et al.*, 1996). Based on this information and the results from this study, they suggest that intra macrophage parasite destruction with garlic may be due to NOS activation as an important effector mechanism in macrophage.

Garlic possesses the ability to inhibit the growth of parasites in the intestines. Garlic has also been used in folk medicine in many parts of the world to treat pinworms, an annoying but generally harmless intestinal parasite. Also, Studies were continued on the pathogenesis of systemic lupus erythematosus, antioxidant effects of garlic/onion oil, development of novel uterine relaxants, pharmacokinetics of rifampicin and ofloxacin and role of angiotensin converting enzyme inhibitors in myocardial stunning ICMR-NIC (2001). Another study was carried out by Kalantari and Salehi (2001) which reported that Garlic oil, as similar to N-acetylcysteine, can eliminate electrophilic intermediates and free radicals through conjugation and reduction reactions. Therefore it protects the liver from toxic doses of acetaminophen. They also observed the protection by the garlic oil. The clearance of the toxic metabolites of the acetaminophen from the liver occurs much faster in immediate treatment with garlic oil (200 mg kg⁻¹).

Results of the present study revealed that probiotic labneh containing garlic and onion oil demonstrated a protective effect and reduced the total worm couple, male and female as well as, ova in liver and intestine in mice infected with *S. mansoni*. Data, also, showed that probiotic labneh containing garlic or onion oil increased the dead oogram in mice infected with *S. mansoni*. It can be concluded that probiotic labneh containing garlic and onion oil may be play a great role as a protective food against infectious diseases such as Schistosomiasis. Furthermore, the garlic oil surpassed the onion oil on their protective effects. Further studies are required to investigate the biochemical and immunological consequences in the humans given such these oils.

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