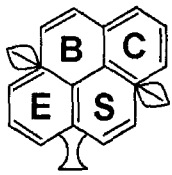

Journal

NUTRITIONAL, BIOLOGICAL AND HISTOLOGICAL EFFECTS OF ANTIOXIDATIVE PROBIOTIC PERMEATE BEVERAGE WITH LEMON JUICE

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ABSTRACT

Forty two (42) adult male albino rats with a body weight ranging from 100- 120 g were used to evaluate the nutritional, biological and histolopathological effects of antioxidative probiotic fermented permeate with lemon, had high viable counts of *Bifidobacterium longum* and *Lactobacillus rhamnosus*, or *Bifidobacterium longum* and *Lactobacillus acidophilus* as compared with hyperlipidemic diets. Antioxidative fermented beverage with lemon had the highest vitamin C content. Also, antioxidative fermented permeate beverage with lemon caused a slight increase in body weight as compared with basal and control positive diets. The percentage of the liver, heart, and spleen weights / body weight significantly decreased and the use of fermented beverage with lemon had no effects on kidney and lung weights / body weight. The use of *Bifidobacterium longum* and *Lactobacillus rhamnosus* as starter culture in production of antioxidative probiotic fermented permeate with lemon had significant effect on relative body and relative organs weights as compared with non fermented and probiotic fermented beverage with *Bifidobacterium longum* and *Lactobacillus acidophilus*.

Serum glucose content, VLDL- cholesterol and LDL-cholesterol, and HDL- cholesterol contents significantly decreased with using of antioxidative fermented permeate beverage with lemon. Serum aspartate amino transferase and serum creatinine contents significantly decreased with the use of antioxidative probiotic

beverage with lemon, as compared with all other diet. Histopathological study showed that the use of antioxidative probiotic beverage with lemon had a great effect on reducing all changes in kidney and liver tissues as compared with hyper lipidemic diets.

Therefore, it could be recommended that the use of antioxidative probiotic beverage with lemon could be great beverage that has great properties on nutritional, biological and histological effects.

Keywords: antioxidative, probiotic, permeate, lemon, beverage, *Bifidobacterium longum*, *Lactobacillus rhamnosus*, biological, serum.

INTRODUCTION

There is an increasing amount of evidence indicating health benefits by consumption of food containing microorganisms like probiotics (Sullivan and Nord, 2002a; Schrezenmeir and DeVrese, 2001). Probiotics were recently redefined by an expert group to 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2001 and 2002; Sullivan and Nord, 2002b; Reid *et al* 2003; Reid and Bruce 2006 and Reid, 2008). A number of clinical trials had been performed to evaluate the effects in the prevention and treatment of gastrointestinal diseases caused by pathogenic microorganisms or by disturbances in the normal microflora (Sullivan and Nord, 2005).

Microorganisms most commonly used as probiotics are lactic acid-producing lactobacilli and bifidobacteria. Both bacterial groups belong to the normal microflora and several strains produce not only lactic acid but also other antimicrobial substances like hydrogen peroxide and bacteriocins (Alvarez-Olmos and Oberhelman, 2001). Probiotic agents further compete with pathogens for microbial adhesion sites and are claimed to modulate the immune response of the host. The specific effects on the immune system are, however, still unclear (Reid *et al* 2001).

Bifidobacteria had long been recognized as probiotic bacteria, had many nutritive, therapeutic and health promoting properties in human and animal intestinal tract (Bezkoravainy, 2001). Probiotic lactobacilli are known to confirm an array of health promoting activbities on their host after either oral administration (deWaard *et al* 2001; de Vrese *et al* 2001 and Oyetayo *et al* 2003). Some of their

beneficial effects include prevention of intestinal infection (Tannock, 1983; Casas and Dobrogosz, 2000), anticarcinogenic activity (Fuller and Gibson, 1997), control of serum cholesterol (Bertazzoni *et al* 2001), enhancement of immunity (Aattouri *et al* 2001), growth enhancement of animals (Baird, 1977 and Chang *et al* 2001).

The mechanism by which these probiotics affects their host and bring and bring about improvement in the gut barrier can be due to competition for adhesion site, production of inhibitory compounds, and rebalancing of disturbed gastrointestinal microbial composition and metabolism (FAO/WHO, 2001 and 2002). Lactobacilli have a long history of use as probiotics without established risk to humans (Naidu *et al* 1999). No pathogenic or virulence properties had being associated with lactobacilli, bifidobacteria or lactococci (Aguirre and Collins, 1993). To further confirm the safety and roles of ingestion of some this probiotics, a study was designed to investigate the effect of *Lactobacillus rhamnosus, Lactobacillus acidophilus* and *Bifidobacterium longum* on the haematological, and some enzymatic activities parameters of healthy and hyper lipidemic rats. A haematological study is a valuable diagnostic tool in evaluating human health (Cheesbrough, 1991). Madsen *et al* (2001) reported that a commercial mixture of probiotic bacteria containing *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbruckii*, *L. plantarum*, and *Streptococcus salivarius* can enhance the epithelial barrier in interluekin-10 knockout mice which serves as a model for inflammatory bowel disease.

The consumption of fruits juice, pulp, nectar, beverage and drinks, in general, had great beneficial effects to health and contributes to the prevention of degenerative processes, particularly lowering incidence and mortality rate of cancer, epithelial cancer and cardiovascular diseases (Hertog *et al* 1993 and Temple, 2000). The National Research Council of the United State recommends consumption of five portions daily of fruits and vegetables, especially citrus fruits. The health benefits are a result of the intake of antioxidants and protective substances like vitamine C and carotenoids, along with bioactive plant secondary metabolites (Slaterry *et al* 2000 and Cara *et al* 2004) and besides ascorbic acid Citrus genus contains flavonon glycoside the most important phenols in the water-soluble fraction (Gil-Lzquierdo *et al* 2001).

The aim of the present study was, therefore, to gain insight into the effect of orally administered antioxidative fermented probiotic permeate beverages with lemon on the nutritional, biological parameters of rats. Moreover, the effect of beverage administration on histological sections of liver and kidney that will bring about health promoting effect in rats was also studied.

MATERIALS AND METHODS

Materials

Fresh milk permeate was obtained from the soft cheese factory of Misr Milk and Food Company, Cairo, in which an ultrafiltration unit CARBOSEP, FRANCE was used. Lemon fruits were obtained from the Egyptian Markets, Cairo, Egypt.

Strains

Bifidobacterium longum ATCC 15707, *Lb. acidophilus* ATCC 20552 and *Lb. rhamnosus* DSMZ 20245 were obtained from the Egyptian Microbial Culture Collection [EMCC] Cairo MIRCEN, Faculty of Agriculture, Ain Shams University.

Active culture of *Bifidobacterium* was freshly propagated in the modified MRS medium supplemented with 0.05% L-cystein and 0.3% lithium chloride (Dave and Shah 1996 and Roy 2001) plates were incubated anaerobically (using gas pack BBL) at 37°C for 48h. While, *Lb. acidophilus* and *Lb. rhamnosus* strains were freshly prepared using MRS medium (De Man *et al* 1960). The flasks or plates were incubated at 37°C for 48h.

Experimental methods

Antioxidative Probiotic Beverage Preparation

Fresh milk permeate was warmed up to 40°C, sucrose (10%) was added. The mixture was heated to 85°C for 30 min, and rapidly cooled to 40°C. The resultant mixture was inoculated with 2% of different single and mixed cultures AB (*Bifidobacterium longum* and *Lactobacillus acidophilus*) and BR (*Bifidobacterium longum* and *Lactobacillus rhamnosus*) starter cultures. The mixtures were incubated at 37°C until pH decreased to 4.8, as rapidly cooled to 5°C. Thereafter, 30% of heat treated Lemon juice; was added. The cooled probiotic fermented beverage was filled into glass bottles (125 ml) and stored at 5°C for 60 days. Samples of antioxidative probiotic

fermented permeate with Lemon juice was taken periodically for animal feeding when fresh and along the storage period.

Vitamins content

Vitamins A, E, C, B_1, B_2 and B_7 were determined as water soluble vitamins content (mg/100g) according to the method described by A.O.A.C. (2007).

Experimental animals

Adult male wistar albino rats (*Rattus norvegicus*) aged 6 to 7 weeks with average weight of 100 to 120g were obtained from the farm of the National Organization for Drug Control and Research, Giza, Egypt

The diet experiment

The composition of diets was casein 15%, corn oil 10%, cellulose 5%, salt mixture 4%, vitamin mixture 1%, and starch 65%. The composition of the salt and vitamin mixtures requirements were given according to A.O.A.C. (2007).

Biological evaluation

Forty two adult male albino rats (western strain) were obtained and housed in seven cages and used throughout this study. They were obtained from the farm of the General Organization of Serum and Vaccine (Helwan farm). At the beginning of the experiment the weight of rats are approximately 95 $\pm$ 5g. The animals were distributed into 6 groups; each group consists of 6 rats. The animals were housed individually in well aerated cages with screen bottoms, at room temperature (25 $\pm$ 2°C). The experimental diets and tap water were supplied. Total body weight of the animals was recorded at the beginning and weekly.

Six rats- initial group was randomly chosen then weighed, and blood sample were withdrawn from retro bulbar venous plexus of each rat according to the procedure of Shermer (1967). Then, rats were sacrificed and organs including liver, kidney, heart, lung and spleen were excised and weighed, liver was stored at -30°C until biochemical analysis. The animals fed on experimental diet control were divided into $^{+}$ve and $^{-}$ve control. The $^{-}$ve control group was fed on free diet. Control ($^{+}$ve) was fed on a diet containing cholesterol (1g) and bile salt (0.250g) for each 100 g was added to raise the cholesterol level in the animal's body.

The other animal groups 1, 2 and 3 were fed on the diets containing cholesterol and bile salt for two weeks. After the cholesterol arose, we began to feed the animal groups 1, 2 and 3 on the diets containing non fermented permeate lemon juice, AB and BR fermented lemon beverages for 6 weeks. At the end of assay the split food was collected and subtracted from the original starting weight of each experimental diet. Composition of experimental and basal diets was prepared according to the method described by A.O.A.C., (2007) and Hegsted *et al* (1941). Also, the animals were anthesized, blood samples were withdrawn from hepatic portal vein (H.P.V.) and the organs (liver, kidney, heart, lungs and spleen) were weighted. The blood serum was biochemiclly estimated (Zhao *et al* 1995).

Blood Sampling

Blood samples from slaughtered animals (30 ml) were collected and immediately divided into aliquots, one containing EDTA (ethylene-diamine tetra acetic acid) as anticoagulant (1 mg/ ml blood) for studying there haemograms of the experimental animals, and the second aliquots containing heparin (10 I.U. /ml) as anticoagulant. Blood was centrifuged (3500 rpm for 15 min) to separate plasma, which was kept in aliquot tubes at -18°C until biochemical assays.

Biochemical analysis

Serum glucose was determined using kits purchased from Biocon (Germany) according to the procedure of Trinder, (1989), total protein was determined according to Henry, (1964), Albumin was determined according to Doumus *et al* (1972).

Total triglycerides was determined according to the method of Fossati and Principe (1982), total cholesterol was determined according to the method of Richmoned (1973), low density lipoprotein (LDL) cholesterol was determined according to Fruchrat, (1982), and high density lipoprotein (HDL) cholesterol determined according to Burstein, *et al* (1970). Liver function enzymes, Alanine amino transferase (ALT or GPT) and Aspartine amino transferase (AST or GOT) were determined according to Reitman and Franked, (1957).

Histological study

Tissue specimens were taken during dressing at the different recorded periods, weighed and divided into two groups, one kept at -18°C until assessing cadmium concentration, the second were

immersed in 10% neutral buffered formaline. The fixed tissue were washed in tap water, dehydrated in a series of alcohol, cleared in xylene then embedded in paraffin blocks. Five micron thickness tissue paraffin sections were obtained then stained by hematoxylin and eosin stain, Carleton, (1967) for histopathological examination. The histological study for male albino rats tissues (liver and kidney) were carried out according to the method described by Bancrofet and Steven (1977).

Statistical analysis

The data were analyzed according to Statistical Analysis System User's Guide **(SAS, 1996)** (SAS Institute, Inc, U.S.A.). Whereas, Duncan multiple ranges was used to analyze the statistical significance.

RESULTS AND DISCUSSION

Vitamins content of antioxidative probiotic beverages with lemon in Table (1) shows that the lemon juice had high vitamin C content as compared with all other determined vitamins. Also, fermentation process had a slight effect on vitamins content in the antioxidative probiotic beverages as compared with non fermented permeate lemon juice mixture. Furthermore, Antioxidative probiotic beverage with AB starter cultures containing the maximum vitamin E content.

Data in Table (2) shows the changes in Body weight in male albino rats feeding with hyper lipidemic diet and different antioxidative fermented permeate beverage for 45 days. The lowest increase in body weight was found with the use of antioxidative fermented permeate beverage in diet had which had the lowest remarkable effect on the body weight as compared with negative and permeate diet. The use of antioxidative fermented permeate beverage with lemon caused a slight increase in body weight, as compared with basal and control positive diets. Schouten *et al* (1985) reported that body weight gain was significantly increased in animals fed on the high cholesterol diet, and this increase may be referred to the higher fat content and thus higher energy density of the diet.

Table (1): Vitamins content of antioxidative probiotic permeate beverages with lemon (mg/100g).

Beverages	Vit. A	Vit. E	Vit.C	Vit.B_1	Vit.B_2	Vit.B_7
Permeate lemon juice mixture	4.0±0.15	5.1±0.2	18.9±0.5	0.6±0.02	6.2±0.4	1.2±0.2
AB antioxidative probiotic permeate beverage	4.85±0.12	5.56±0.15	21.3±0.4	0.8±0.01	9.8±0.2	3.6±0.4
BR antioxidative probiotic permeate beverage	4.2±0.22	5.4±0.14	19.1±0.5	0.7±0.02	9.01±0.3	3.4±0.2

*Mean ± S.D

Table (2): Body weight changes in male albino rats feeding with hyper lipidemic diet and different antioxidative probiotic permeate beverages for 45 days

Beverages	Body weight (g)		$\bar{X}_f - \bar{X}_I$
	Initial ($\bar{X}_I$)	Final ($\bar{X}_f$)	
Control (-)	86.6	115.3	28.7[a]
Control (+)	86.5	108.6	22.1[b]
Permeate lemon juice mixture	99.7	112	12.3[d]
AB antioxidative probiotic permeate beverage	102	124	22[b]
BR antioxidative probiotic permeate beverage	94.6	115.2	20.7[c]
Se- ACE	92.3	115	22.7[b]

n = 6 rats

Table (3) presents the percentage of relative organs weight of male albino rats feeding with Hyperlipidemic diet and different antioxidative probiotic permeate beverages for 45 days. It could be noticed that there are an increase in relative weight of liver in rats fed hyperlipidemic diet, as compared with the negative control diet. Induced rats with AB and BR antioxidative probiotic permeate beverages showed a significant ($P \leq 0.05$) reduction in the liver relative weight and kidney increased in rats fed on both as compared with both control diets. The decreased on liver relative weight could be useful in liver function. On the other hand, heart relative weights significantly decreased in rats fed on both antioxidative probiotic permeate beverages with lemon in a diet. While, AB and BR antioxidative probiotic permeate beverages had no remarkable effect on the

percentage of Heart and Lung relative weights as compared with both control diets.

The data are in agreement with those found by Aboderin and Oyetayo (2006) who observed that there was a significant increase ($P<0.05$) in the weight gained by rats fed *Lactobacillus plantarum* when compared to the control. *Lactobacillus plantarum* is safe and it has immunostimulatory effect and can also improve the performance of rats in terms of weight gain. Khalon *et al* (1997) found an increase in relative weight of liver in rats fed hyper lipidemic diet. And this increase might be due to fat deposition mainly in liver. Furthermore, the main effect of hyper lipidemic diet was a sever liver with an accumulation of both cholesterol and triglycerides (Robins *et al* 1994). Rasic and Kurman (1983) reported that the immune system and anticarcinogenic activity enhanced using diets containing probiotic bacteria (*Bifidobacterium* sp.).

Table (3): percentage of relative weight organs (weight / body weight) of male albino rats feeding with Hyperlipidemic diet and different antioxidative probiotic permeate beverages for 45 days.

Beverages	Liver	Kidney	Heart	Lung	Spleen
Initial weight	4.63 ± 1.832	0.90 ±0.038	0.45 ± 0.016	1.13 ± 0.019	0.47 ± 0.025
Control (-)	4.09 ± 0.140	0.93± 0.027	0.48 ± 0.018	1.01 ± 0.167	0.46 ± 0.008
Control (+)	4.72 ± 0.077	0.87 ±0.012	0.49 ± 0.015	1.05 ± 0.021	0.49 ± 0.015
Permeate lemon juice mixture	4.63 ± 1.851	0.90 ± 0.038	0.49 ± 0.012	1.08 ± 0.018	0.38 ± 0.010
AB antioxidative probiotic permeate beverage	4.98 ± 0.195	1.00± 0.025	0.47± 0.016	1.13± 0.017	0.39± 0.021
BR antioxidative probiotic permeate beverage	4.95 ± 0.046	0.98± 0.038	0.49 ± 0.015	1.13 ± 0.029	0.45 ± 0.017
Se- ACE	4.37± 0.112	0.91 ± 0.020	0.49 ± 0.006	1.11 ± 0.027	0.45 ± 0.014

*Mean ± S.D n = 6 rats

Data in Table (4) presents that, the serum glucose content significantly decreased with the use of AB and BR antioxidative probiotic permeate beverages with lemon as diets. Whereas, the permeate lemon juice mixture caused a significant increase in serum glucose content as compared with male albino rats feeding with control hyperlipidemic diet. Generally, BR antioxidative probiotic permeate beverage caused significant decrease in serum glucose content as compared with all other treatments.

On the other hand, serum cholesterol and serum triglycerides contents significantly decreased with the use of both antioxidative probiotic permeate beverages with lemon as compared with all other diets. While, the rats fed on BR antioxidative probiotic permeate beverages had the lowest increase in serum cholesterol content. Generally, the significant decrease in serum glucose content might be due to the use of probiotics (*Bifidobacterium longum, Lactobacillus acidophilus* and *Lactobacillus rhamnosus*) as starter cultures in manufacture of the probiotic beverage with lemon, and the increase may be due to the sugar content (10%) antioxidative fermented lemon permeate beverage. Also, this increase in serum cholesterol had no diverse effect on rat's health. Furthermore, deconjugated bile salts do not stimulate the absorption of cholesterol and other lipids from the small intestine as well as do conjugated bile salts. Thus, the more bile salts excreted, the more cholesterol is removed from the body. Serum triglycerides were also lowered as a result of antioxidative probiotic permeate with lemon juice containing both AB and BR probiotic starters treatments, without affecting the structure and relative weight of the liver. This suggests that the hypolipemic effect of the bacteria may not be due to a redistribution of lipids from the plasma to the liver, but rather to decreased intestinal absorption of lipids or increased lipid catabolism (Taranto *et al* 1998 and Nguyen *et al* 2007).

Serum HDL-cholesterol, VLDL- cholesterol and LDL-cholesterol contents of male albino rats feeding with hyperlipidemic diet and different antioxidative probiotic permeate beverages for 45 days are presented in Table (5). The data shows that, the HDL-cholesterol content slightly decreased as compared with, control, and permeate lemon juice mixture as diets. On the contrary, the permeate lemon juice mixture had the highest significant effect on HDL-cholesterol content. Whereas, serum VLDL- cholesterol and LDL-cholesterol contents significantly decreased with the use of antioxidative probiotic permeate lemon beverages, followed by the use of permeate lemon diets as compared with all other diets. On the other hand the control negative diet caused a significant increase in VLDL- cholesterol and LDL- cholesterol contents. The decrease in VLDL- cholesterol and LDL- cholesterol contents with the use of antioxidative probiotic permeate lemon beverages may be due to the high viable content of *Bifidobacterium longum, Lactobacillus*

acidophilus and *Lactobacillus rhamnosus* used as probiotic starter cultures in beverage fermentation.

Bifidobacteria had remarkable effect for cholesterol assimilation and bile salts, because of their bile salt-deconjugating activity and cholesterol precipitation (Harrison and Peat 1975; Hoover 1993; Hughes and Hoover 1991; Klaver and Van der Meer 1993; Mann and Spoerry 1974 and Rasic *et al* 1992). High serum cholesterol concentration is associated with the development of coronary heart disease (Usman and Hosono 1999 and 2000).

Table (4): Serum glucose, serum total cholesterol and serum triglycerides contents of male albino rats feeding with hyperlipidemic diet and different antioxidative probiotic permeate beverages for 45 days.

Beverages	Initial ($\bar{X}_I$)	Final ($\bar{X}_f$)	$\bar{X}_f - \bar{X}_i$	Differences with C+
	Serum glucose (mg/dl)			
Control (-)	76.55 ± 1.7	88.21 ± 4.26	11.7	1.3
Control (+)	77.0 ± 1.17	89.96 ± 3.44	13.0	-
Permeate lemon juice mixture	77.1 ± 1.18	96.1 ± 4.6	19.0	6.6*
AB antioxidative probiotic permeate beverage	76.50 ± 1.72	76.36 ± 5.5	29.86	-0.14*
BR antioxidative probiotic permeate beverage	74.9 ± 1.8	73.02 ± 3.6	3.12	-9.88**
Se-ACE	77.1 ± 1.21	90.1 ± 3.6	13.0	0
	Serum cholesterol (mg/dl)			
Control (-)	79.5 ± 2.26	87.54 ± 1.5	8.0	37.39
Control (+)	78.3 ± 2.25	123.69 ± 1.8	45.39	-
Permeate lemon juice mixture	76.1 ± 2.31	109.35 ± 1.5	33.25	12.14*
AB antioxidative probiotic permeate beverage	78.3 ± 2.10	101.45 ± 1.5	23.15	22.24**
BR antioxidative probiotic permeate beverage	77.6 ± 2.32	84.53 ± 1.6	6.93	38.45**
Se-ACE	78.7 ± 2.29	82.23 ± 2.4	3.53	41.86**
	Serum triglycerides (mg/dl)			
Control (-)	69.44 ± 1.16	71.59 ± 5.0	2.15	15.88
Control (+)	67.94 ± 1.19	85.99 ± 2.7	18.0	-
Permeate lemon juice mixture	68.10 ± 1.22	76.86 ± 4.5	8.76	9.24*
AB antioxidative probiotic permeate beverage	71.02 ± 1.19	67.24 ± 4.5	3.78	14.22*
BR antioxidative probiotic permeate beverage	67.88 ± 1.21	56.76 ± 2.7	-11.12	29.12
Se-ACE	69.29 ± 1.19	75.99 ± 2.7	6.7	11.3*

** highly significantly difference ($p < 0.1$ %) * significantly ($p < 0.5$ %)
mean ± SD

Many studies have reported the ability of *Lactobacillus acidophilus* (Gilliland *et al* 1985 and Usman and Hosono 1999) and bifidobacteria (Dambekodi and Gilliland 1998) to assimilate cholesterol from laboratory media. Thus, both types of bacteria may have the potential to reduce serum cholesterol in humans.

Table (5): Serum HDL-cholesterol, VLDL- Cholesterol and LDL-Cholesterol contents of male albino rats feeding with hyperlipidemic diet and different antioxidative probiotic permeate beverages for 45 days

Beverages	Initial ($\bar{X}_I$)	Final ($\bar{X}_f$)	$\bar{X}_f - \bar{X}_i$	Differences with C+
	HDL- Cholesterol (mg/dl)			
Control (-)	34.60 ± 0.57	48.47 ± 1.36	13.87	0.01
Control (+)	36.30 ± 0.55	50.18 ± 2.4	13.88	-
Permeate lemon juice mixture	34.88 ± 0.54	39.38 ± 2.9	4.5	-9.38*
AB antioxidative probiotic permeate beverage	33.92 ± 0.52	48.90 ± 1.0	14.98	- 1.1
BR antioxidative probiotic permeate beverage	35.60 ± 0.55	47.88 ± 1.14	12.28	-1.60
Se- ACE	35.71 ± 0.52	37.47 ± 0.9	1.76	-12.21**
	VLDL- Cholesterol (mg/dl)			
Control (-)	13.9±0.1	14.32±0.6	0.42	3.18
Control (+)	13.6±0.2	17.2±0.2	3.6	-
Permeate lemon juice mixture	13.6±1.0	15.4±0.8	1.8	-1.8*
AB antioxidative probiotic permeate beverage	14.2±0.5	13.45±0.8	-0.75	-4.35*
BR antioxidative probiotic permeate beverage	13.58±0.6	11.35±0.9	-2.23	-5.83*
Se- ACE	13.86±0.4	15.2±1.0	1.34	2.26*
	LDL- Cholesterol (mg/dl)			
Control (-)	31.0 ± 1.77	24.75 ± 1.1	-6.25	21.66
Control (+)	28.4 ± 1.69	56.31 ± 3.3	27.91	-
Permeate lemon juice mixture	27.62 ± 1.51	54.57 ± 2.5	26.95	-0.96
AB antioxidative probiotic permeate beverage	30.18 ± 1.36	39.1 ± 1.6	8.92	-18.99
BR antioxidative probiotic permeate beverage	28.42 ± 1.42	25.3 ± 1.07	-3.12	-31.03
Se- ACE	29.13 ± 1.39	29.53 ± 3.3	0.4	-27.51

** highly significantly difference ($p < 0.1$ %) * significantly ($p < 0.5$ %)
mean ± SD

Table (6): Serum aspartate amino transferase (AST), Serum alanine amino transferase (ALT) and Serum creatinine contents of male albino rats feeding with hyperlipidemic diet and different antioxidative probiotic permeate beverages for 45 days.

Beverages	Initial ($\bar{X}_1$)	Final ($\bar{X}_f$)	$\bar{X}_f - \bar{X}_i$	Differences with C +
	AST (u/l)			
Control (-)	58.44 ± 3.67	64.61 ± 2.88	6.17	5.86
Control (+)	54.44 ± 3.65	66.47 ± 2.6	12.03	-
Permeate lemon juice mixture	55.17 ± 3.2	57.07 ± 2.9	1.9	10.13**
AB antioxidative probiotic permeate beverage	56.71 ± 3.2	57.54 ± 1.8	0.83	11.2**
BR antioxidative probiotic permeate beverage	56.16 ± 3.11	53.25 ± 2.8	-2.91	-14.94**
Se- ACE	55.17 ± 3.21	65.43 ± 2.1	10.26	+1.77
	ALT (u/l)			
Control (-)	17.86 ± 0.91	22.23 ± 1.66	4.37	29.27
Control (+)	17.87 ± 0.89	51.51 ± 1.14	33.64	-
Permeate lemon juice mixture	17.10 ± 0.89	17.62 ± 1.16	0.52	33.12**
AB antioxidative probiotic permeate beverage	17.99 ± 0.93	24.01 ± 1.69	6.02	27.62**
BR antioxidative probiotic permeate beverage	15.03 ± 0.95	16.12 ± 0.45	1.09	32.55**
Se- ACE	17.01 ± 0.92	23.37 ± 0.93	6.36	27.28**
	Serum creatinine (u/l)			
Control (-)	0.28 ± 0.015	0.48 ± 0.01	0.2	0.04
Control (+)	0.26 ± 0.012	0.50 ± 0.03	0.24	-
Permeate lemon juice mixture	0.25 ± 0.011	0.34 ± 0.007	0.09	0.15
AB antioxidative probiotic permeate beverage	0.24 ± 0.012	0.32 ± 0.009	0.08	0.16
BR antioxidative probiotic permeate beverage	0.28 ± 0.12	0.26 ± 0.006	-0.02	-0.26
Se- ACE	0.25 ± 0.018	0.64 ± 0.023	0.39	0.15

** highly significantly difference ($p < 0.1$ %) * significantly ($p < 0.5$ %)
mean ± SD

Besides the hypocholesterolemic action, Akalin *et al* (1997) also reported a higher number of faecal lactobacilli and fewer coliforms in mice that received acidophilus yoghurt than in those that received plain yoghurt, thus indicating that *L. acidophilus* established itself more effectively in the murine intestinal tract than did *L. bulgaricus* in the yoghurt diet. Aattouri *et al* (2001) had earlier reported that oral ingestion of lactic acid bacteria by rat's increases lymphocyte proliferation and interferon a production. Nguyen *et al* (2007) found that, the serum cholesterol and triglycerides were respectively 7 and

10% lower in the group fed *L. plantarum* PH04, while no any significant differences ($P \leq 0.05$) in body weight.

As shown in Table (6), the use of antioxidative fermented beverage with lemon had significant effects on serum aspartate amino transferase and serum creatinine contents. While, the serum alanine amino transferase content slightly increased but more less than the control with hyperlipidemic diet this caused the highest increase.

Serum aspartate amino transferase and serum creatinine contents significantly decreased with the use of antioxidative fermented beverage with lemon as compared with all other diet. The decreases in all serum aspartate amino transferase, serum alanine amino transferase and serum creatinine contents with the use of antioxidative fermented beverage with lemon in diet might be due to the high effect of antioxidants, and high viable content of *Bifidobacterium longum* and *Lactobacillus rhamnosus*.

A few experimental animal studied were successful in positively attributing cholesterol lowering properties to *L. acidophilus* contained in dairy products administered as acidophilus yoghurt to pigs (Jones *et al* 1985) and mice (Akalin *et al* 1997). Marteau and Rambaud (1993) reported that significant reduction in serum cholesterol during consumption of large doses (680±5000 mL/d) of certain probiotic fermented dairy products. The theory assumed for the hypocholesterolemic effect was the presence of suchorganic acids as uric, orotic and hydroxymethylglutaric, which actually inhibit cholesterol synthesis (Fernandes *et al* 1987 and Gomes and Malcata, 1999). The mechanisms by which fermented milk products are able to reduce serum cholesterol have not to date been fully demonstrated.

Fuller and Gibson (1997), reported that probiotics had been used as growth promoters due to their ability to suppress the growth and activities of growth depressing microflora and their ability in enhancing absorption of nutrients through the production of digestive enzymes. Oyetayo and Osho (2004), found that the *Lactobacillus plantarum* shows that it can antagonize both pathogenic and food spoilage bacteria, adhere to the ileal epithelial cell of rats, hepatoproctective ability by lowering serum aminotranferase level and anti-cholesrerolaemic effect.

In vitro studies by Gilliland (1990), showed that bifidobacteria and *L. acidophilus* are able to utilize cholesterol in growth media, both by assimilation and precipitation with deconjugated bile salts under

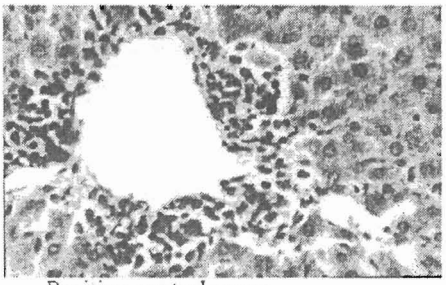
acidic conditions. Their suggestion that the presence of bile salts is a prerequisite for cholesterol assimilation by bifidobacteria was confirmed by the observations of Klaver and van der Meer (1993), and more recently by those of Tahri *et al* (1995 and 1997) who went one step further and investigated the nature of the bile salts; according to these authors (Tahri *et al* 1997) assimilation of cholesterol was higher in the presence of trihydroxyconjugated bile salts than dihydroxyconjugated bile salts. The aforementioned bacterial assimilation of cholesterol in the intestine may reduce its absorption from the digestive tract into the blood system.

Histopathological study

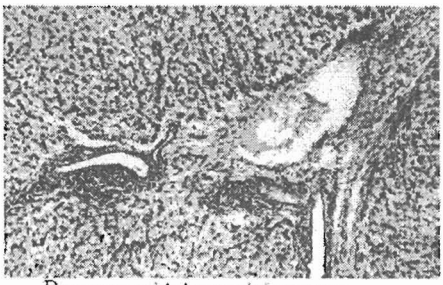
Photos (1 and 2) expose that the Liver of rat in negative control group showing focal mononuclear leucocytes inflammatory cells aggregation dilatation of central as well as portal vein associated with diffuse Proliferation of kupffer cells. While, Liver of rat in positive control group identify the mononuclear leucocytes inflammatory cells infiltration surrounding the central vein.

Liver of rat in permeate group showing massive number of inflammatory cells aggregation in the portal area associated with degeneration in the hepatocytes. On the contrary, Liver of rat in antioxidative probiotic permeate with lemon juice group showing periodical mononuclear leucocytes inflammatory cells infiltration surrounding the bile duct associated with fibrosis and dilatation of the portal vein in the portal area. Whereas, Liver of rat in permeate with lemon juice group showing focal mononuclear leucocytes inflammatory cells aggregation in the hepatic tissue parenchyma.

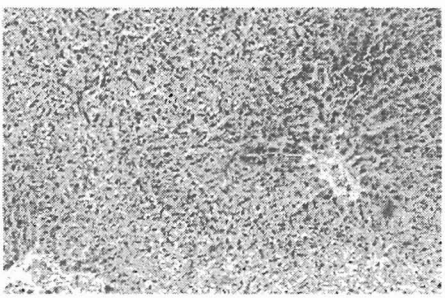
The data in Photo (2) illustrates the Kidney of rat in positive control group showing focal extra vacation of red blood cells in between the degenerated renal tubules at the corticomedullary junction. While, Kidney of rat in permeate lemon juice group mononuclear leucocytes inflammatory cells infiltration in the vascular tissue as well as between the renal tubules and glomeruli in manner. On the other hand, kidney of rat in fermented permeate with lemon juice group showing degeneration in the renal tubules at the corticomodullary junction. Kidney of rat in permeate lemon juice group showing swelling and vacuolation in the endothelial cells living the glomerular tact of the glumeruli.



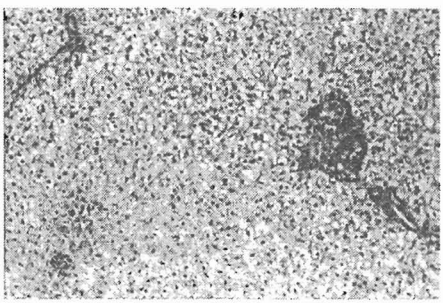
Positive control group



Permeate with lemon juice group

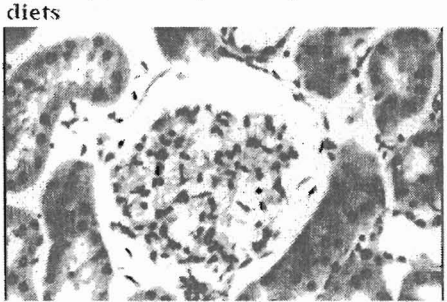


BR antioxidative probiotic permeate beverage group

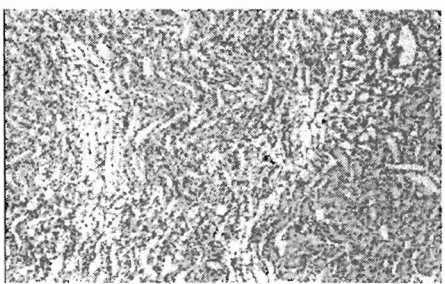


AB antioxidative probiotic permeate beverage group

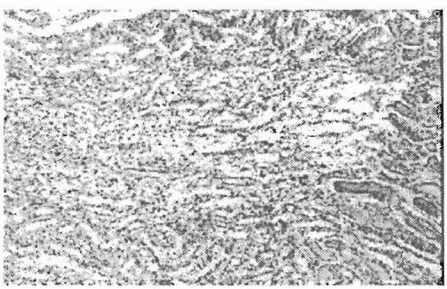
Photo (1): Histopathological sections in Liver of rat groups fed on different diets



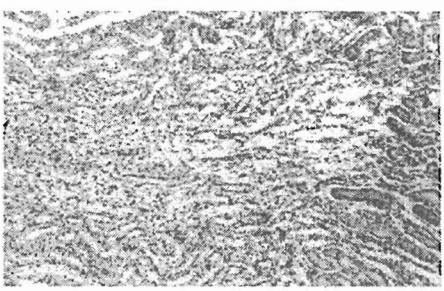
Positive control group



Permeate lemon juice group



AB antioxidative probiotic permeate beverage group



BR antioxidative probiotic permeate beverage group

Photo (2): Histopathological sections in Kidney of rat groups fed on different diets.

Evaluation of pathogenicity is one important factor of probiotic safety studies (Marteau *et al* 1997 and Zhou *et al* 2000), the indicators for which include splenomegaly and hepatomegaly. None of these morphological changes was noted as a result of antioxidative probiotic permeate with lemon juice containing both AB and BR probiotic starters treatments, nor were these significant differences in the visceral weight indices of the lymph nodes, spleen, or liver.

Therefore, the antioxidative fermented beverage with lemon could be recommended as beverage has great properties of nutritional, biological and histological effects.

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التأثيرات التغذوية والحيوية والهستولوجية لمشروب علاجى متخمر من راشح اللبن وعصير الليمون

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تم استخدام راشح اللبن فى صناعة مشروب مرتفع فى محتواه من مضادات الأكسدة بالإضافة إلى ارتفاع محتواه من البكتريا ذات الخواص الوقائية (المدعمات الحيوية) وقد استخدم نوعان من البادئات هما (BR) ويحتوى السلالات *Bifidabacterium longum* و *Lactobacillus rhamnosus* والثانى (AB) ويحتوى على *Bifidabacterium longum* و *Lactobacillus acidophilus* حيث تم تلقيح الراشح بعد معاملته حراريا"(90°م / 15 ق ثم التبريد حتى 40°م) بالخلطات المختلفة من بادئ *Bifidabacterium longum* و *Lactobacillus rhamnosus* والبادئ الأخر من *Bifidabacterium longum* و *Lactobacillus acidophilus*).

وبعد إنتهاء عملية التخمر (عند pH 4.8) تم إضافة عصير الليمون المعامل حراريا (80°م / 20 ق) ومحلى (نسبة السكر 10%) بنسبة 30% ثم التعبئة تحت شروط التعقيم فى زجاجات معقمة محكمة الغلق والتخزين بالثلاجة لمدة 3 شهور وأجرى تقييمها بيولوجيا باستخدام فئران التجارب Male albino rats (100-120جم) المغذاة على عليقة مرتفعة فى محتواها من الدهون لمعرفة تأثير هذه الخلطات على وزن الجسم ومكونات الدم ووظائف الكبد والكلى والتغيرات فى أنسجة الكبد والكلى كما تم اخذ عينات من الدم فى بداية التجربه ونهايتها وتم فصل سيرم الدم وإجراء التحاليل البيوكيميائيه به لتقدير الجلوكوز- لبيدات الدم – إنزيمات الكبد والكرياتينين، وفي نهاية التجربة تم وزن الفئران ثم ذبحها وفصل أعضائها وهى (الكبد، الكلى ، القلب ، الرئه ،والطحال) ووزنها وإعداد التقييم الهستولوجي لها.

ووجد أن استخدام هذه المشروبات المرتفعة فى مضادات الأكسدة ذات القيمة الحيوية العالية قد أدى إلى خفض دهون الدم وتحسين وظائف الدم والكلى ووزن الجسم. كما أدت إلى نتائج جيدة فى مقاومة أى زيادة فى الكوليستيرول الكلى والكوليسترول الردئ بنوعيه والجلسريدات الثلاثية وفى نفس الوقت زيادة قيمة الكوليسترول الجيد كل ذلك حتى مع تناول أغذية عالية فى محتواها من الدهون وكذلك مقاومة أى تغيرات تحدث فى الكبد والكلى نتيجة تناول هذه المشروبات .

كما تم إجراء الفحص الهستوباثولوجى لأنسجة بعض أعضاء الفئران (لأنسجة الكبد والكلى) المأخوذة للتقييم البيولوجى لمعرفة تأثير هذه الخلطات على أنسجة هذه الفئران فى المجموعات المختلفة ووجد أن هذه المشروبات تقلل من الأثار الناتجة عن تغذية الفئران بالعليقة المرتفعة فى الدهون على أنسجة الفئران.

وبالتالى يمكن التوصية بتناول المشروب المرتفع فى محتواه من مضادات الأكسدة بالإضافة إلى إرتفاع محتواه من البكتريا ذات الخواص الوقائية (المدعمات الحيوية) بنكهة الليمون.