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EVALUATION THE EFFECT OF *LACTOBACILLUS ACIDOPHILUS* AND *BIFIDOBACTERIUM* AT LIVER INJURIES OF ISONIAZI-INDUCED RATS

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ABSTRACT

Tuberclosis is the infection disease with bacterial paternity that can affect different part of the body such as respiratory system, derm and digestive system. Isonaizid is a important drug in first line of tuberclusis treating that creates more liver injuries.

The aim of this study was investigation the effect of Lactobacillus acidophilus and bifidobacterium at liver tissue injuries that are created by Isoniazid.

In this study, 60 Sprague – dally male rats in 4 groups with 15 rats per each group were considered. One of groups (non treated group, NT) was without any injection and only with physiological diatery. One another group was Isoniazid group that were injected Isoniazid with dose of 50 mg/kg IP (I-1,I-2 and I-3).The third gRoup was probiotics with Isoniazid that were receiving lactobacillus acidophilus in concentration of 2×10^9 CFU/ml,Bifidobacterium in concentration of 1×10^8 CFU/ml (ILB-1,ILB-2 and ILB-3) and Isoniazid with dose of 50 mg/kg.The last group was Probiotics only receiving (LB-1,LB-2 and LB-3).In each groups at

the end of 1th,2th and 3th week, the blood was taken after anesthesia. One rat was selected randomely at the end of weeks and the liver was removed to pathologically studies.

Isoniazid significantly increased the values of Alanine amino transferase and Aspartat trans aminase (p=0.02),Direct, indirect and Total Billirubin (p=0.04) and Creatin phospho kinase (p=0.01) after three week while Lactobacillus acidophilus and Bifidobacterium with Isoniazid significantly decreased the values of Alanine amino transferase and Aspartat trans aminase (p=0.03),Direct and indirect Billirubin (p=0.04),Total Billirubin (p=0.03), Super oxide dismutase and malondealdehyde (p=0.02) and creatin phosphor kinase (p=0.03) after three week. Isoniazid significantly decreased the values of Albomin and Total protein (p=0.01).Administration of these probiotics with Isoniazid significantly increased the values of Albomin, Total protein (p=0.03) and Glothatione peroxidase (p=0.03) after three week. In this study Isoniazid created some damagefull effects in liver such as hyperemia,WBC infiltration, hydropic degeneration and wide destination of inter hepatocytes specially at the 3th week after Isoniazid consumption but the probiotics significantly decreased these damagefull changes.

Tuberclusis is the infection disease that has worldwide expanding and in sensitive regions it can be endemically. Military forces are attemping contrast with this disease in these regions and this conditions leading to increasing the risk of this disease. Isoniazid has the damagefull effects in liver and lactobacillus acidophilus with Bifidobacterium have the prepairing effects in liver. From these results we conclude that these probiotics significantly decrease the damagefull effects of Isoniazid in liver tissue. The results of this study are usefull at the future researches in human cases and finally in military forces.

INTRODUCTION

Isoniazid (Laniazid, Nydrazid), also known as isonicotinylhydrazine (INH), is an organic compound that is the first-line medication in prevention and treatment of tuberculosis. Isoniazid is a prodrug and must be activated by a bacterial catalase-peroxidase enzyme that in M. tuberculosis is called KatG. KatG couples the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex. This complex binds tightly to the enoyl-acyl carrier protein reductase known as InhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase (1). This process inhibits the synthesis of mycolic acid, required for the mycobacterial cell wall. A range of radicals are produced by KatG activation of isoniazid, including nitric oxide, which has also been shown to be important in the

action of another antimycobacterial prodrug PA-824. Hepatotoxicity of INH is by nitrogen group in its chemical structure, as it is metabolized in the liver and gets converted to an ammonium molecule, which causes hepatitis. Isoniazid reaches therapeutic concentrations in serum, cerebrospinal fluid, and within caseous granulomas (2, 3). It is metabolized in the liver via acetylation. Two forms of the enzyme are responsible for acetylation, so some patients metabolize the drug more quickly than others. Hence, the half-life is bimodal, with peaks at one and three hours in the US population. The metabolites are excreted in the urine. Doses do not usually have to be adjusted in case of renal failure.Adverse reactions include rash, abnormal liver function tests (4), hepatitis, sideroblastic anemia (5), high anion gap metabolic acidosis (6), peripheral neuropathy (7), mild central nervous system (CNS) effects (8), drug interactions resulting in increased phenytoin (Dilantin) or disulfiram (Antabuse) levels (9), intractable seizures (status epilepticus) and drug-induced lupus erythematosus (10,11).Peripheral neuropathy and CNS effects are associated with the use of isoniazid and are due to pyridoxine (vitamin B_6) depletion (12), but are uncommon at doses of 5 mg/kg. Persons with conditions in which neuropathy is common (e.g., diabetes, uremia, alcoholism, malnutrition, and HIV infection), as well as pregnant women and persons with a seizure disorder, may be given pyridoxine (vitamin B_6) (10–50 mg/day) with isoniazid (13).Hepatotoxicity of INH is by nitrogen group in its chemical structure, as it is metabolized in the liver and gets converted to an ammonium molecule, which causes hepatitis.Hepatotoxicity can be avoided with close clinical monitoring of the patient, to be specific, nausea, vomiting, abdominal pain, and appetite. Isoniazid is metabolized by the liver mainly by acetylation and dehydrazination (14, 15). The N-acetylhydrazine metabolite is believed to be responsible for the hepatotoxic effects seen in patients treated with isoniazid. The rate of acetylation is genetically determined. Approximately 50% of blacks and Caucasians are slow inactivators; the majority of Inuit and Asians are rapid inactivators. The half-life in fast acetylators is one to two hours, while in slow acetylators, it is two to five hours. Elimination is largely independent of renal function, but the half-life may be prolonged in liver disease. The rate of acetylation has not been shown to significantly alter the effectiveness of isoniazid (16). However, slow acetylation may lead to higher blood concentrations

with chronic administration of the drug, with an increased risk of toxicity. Fast acetylation leads to higher blood levels of the toxic metabolite acetylisoniazid and thus to an increase in toxic reactions - hepatitis which is 250 times more common than in slow acetylators. Isoniazid and its metabolites are excreted in the urine with 75 to 95% of the dose excreted in 24 hours (17). Small amounts are also excreted in saliva, sputum, and feces. Isoniazid is removed by hemodialysis and peritoneal dialysis. Headache, poor concentration, weight gain, poor memory, and depression have all been associated with isoniazid use. All patients and healthcare workers should be aware of these serious adverse effects, especially if suicidal thinking or behavior are suspected. INH is known to reduce cytochrome P450 and in theory promotes the efficacy of contraceptives (18). Therapy is often combined with rifampin. Rifampin increases the P450 enzyme and also can reduce the efficacy of contraceptives. Alternative means of birth control should be used when taking these medications.As previously mentioned, isoniazid is associated with pyridoxine deficiency. Pyridoxyl phosphate (derivative of pyridoxine, i.e. Vitamin B6) is required for d-aminolevulinic acid synthase, the enzyme responsible for the rate-limiting step in heme synthesis. As such, isoniazid-induced pyridoxine-deficiency leads to insufficient heme formation in early red blood cells, leading to sideroblastic anemia.Hepatotoxicity can be avoided with close clinical monitoring of the patient, to be specific, nausea, vomiting, abdominal pain, and appetite. Isoniazid is metabolized by the liver mainly by acetylation and dehydrazination. The N-acetylhydrazine metabolite is believed to be responsible for the hepatotoxic effects seen in patients treated with isoniazid. The rate of acetylation is genetically determined. Approximately 50% of blacks and Caucasians are slow inactivators; the majority of Inuit and Asians are rapid inactivators. The half-life in fast acetylators is one to two hours, while in slow acetylators, it is two to five hours. Elimination is largely independent of renal function, but the half-life may be prolonged in liver disease. The rate of acetylation has not been shown to significantly alter the effectiveness of isoniazid. However, slow acetylation may lead to higher blood concentrations with chronic administration of the drug, with an increased risk of toxicity. Fast acetylation leads to higher blood levels of the toxic metabolite acetylisoniazid and thus to an increase in toxic reactions - hepatitis which is 250 times more common than in slow acetylators.

Probiotics are microorganisms that some have claimed provide health benefits when consumed. Probiotics are under considerable research, as the concept holds promise for human health and well-being, and corresponding commercial opportunities. Protection of consumers requires health claims to be confirmed with sufficient scientific evidence. Overall scientific demonstration of probiotic effects requires defining a healthy microbiota and interactions between microbiota and host, and the difficulty to characterize probiotic effectiveness in health and disease. Recent developments of high-throughput sequencing technology and the consequent progresses of metagenomics represent a new approach for the future of probiotics research. Studies are examining whether probiotics affect mechanisms of intestinal inflammation, diarrhea, urogenital infections or allergies. Through 2012, however, in all cases proposed as health claims to the European Food Safety Authority, the scientific evidence remains insufficient to prove a cause and effect relationship between consumption of probiotic products and any health benefit (19). Research into the potential health effects of supplemental probiotics has included the molecular biology and genomics of Lactobacillus in immune function (20), cancer, and antibiotic-associated diarrhea (21), travellers' diarrhea (22), pediatric diarrhea, inflammatory bowel disease and irritable bowel syndrome (23). Testing of a probiotic applies to a specific strain under study. The scientific community cautions against extrapolating an effect from a tested strain to an untested strain. There is very little evidence to support claims that probiotic dietary supplements have any health benefits. One expert reasoned that preliminary clinical results exist for some applications, such as treating diarrhea, but wider health benefits claimed by probiotic proponents lack plausibility since the body's "ecosystem" is sufficiently complex that adding a few bacteria is unlikely to have the claimed effect. Accordingly, "the alleged health benefits of probiotics are often an example of spin".

Lactobacillus acidophilus (whose name, from Latin, means 'acid-loving milk-bacterium') is a species of bacteria in the genus Lactobacillus. L. acidophilus is a homofermentative species, fermenting sugars into lactic acid, and grows readily at rather low pH values (below pH 5.0) and has an optimum growth temperature of around 37 °C (99 °F) (24). L. acidophilus occurs naturally in the human and animal gastrointestinal tract and mouth. Some strains of L. acidophilus may be considered to have probiotic characteristics (25). These

strains are commercially used in many dairy products, sometimes together with Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus in the production of acidophilus-type yogurt (26). Some strains of L. acidophilus have been studied extensively for health effects. The Mayo Clinic publishes a list of disorders for which L. acidophilus has been tested, grading the evidence for each use from strong evidence of effectiveness, through unclear, down to strong evidence of ineffectiveness. According to the list there is good (rather than strong) evidence supporting the use of L. acidophilus or yogurt enriched with it for the treatment of some vaginal infections; effectiveness for other conditions ranges from unclear to fair negative evidence (27).Some L. acidophilus strains may be able to survive gastrointenstinal transit, being resistant to bile, low pH, and digestive enzymes (28). They may then be able to adhere to human epithelial cell lines and human intestinal mucus. A blend of bacterial strains including L. acidophilus NCFM decreased the incidence of pediatric diarrhea. L. acidophilus led to a significant decrease in levels of toxic amines in the blood of dialysis patients with small bowel bacterial overgrowth. At adequate daily feeding levels, L. acidophilus may facilitate lactose digestion in lactose-intolerant subjects. A University of Nebraska study found that feed supplemented with L. acidophilus L1 and fed to cattle resulted in a 61% reduction of Escherichia coli O157:H7 (29). Research has indicated L. acidophilus may be helpful reducing serum cholesterol levels. Antibiotics taken orally will kill beneficial, as well as harmful, bacteria, including L. acidophilus. After a therapy that includes antibiotics, patients are occasionally instructed to take an L. acidophilus treatment in order to recolonize the gastrointestinal tract. To that effect, L. acidophilus is often sold in health stores in pill or powder form as a nutritional supplement, as well as being available in many yogurts. A part of the claims in favor of such treatment refer to attaining a better digestion thanks to a recovered normal intestinal flora. L. acidophilus LA-5 produces bacteriocin CH5 that is both antibacterial and inhibitory against certain yeasts and molds and is effective against both Salmonella typhimurium and Campylobacter jejuni (30). It has been shown to improve bowel regularity and has been shown to have a preventative effect against traveller's diarrhea, as well as antibiotic-related bowel issues.[5]Because of its relation to gut-associated lymphoid tissue (GALT), L. acidophilus LA-5 has been associated with positive effects on the immune system such as increased cytokine,

phagocytic activity and antibody production, as well as phagocytosis of Salmonella, and L. acidophilus NCFM has even been shown to reduce incidence of symptoms of fever, cough and runny nose. Anti-inflammatory effects have also been observed in people consuming L. acidophilus NCFM. Additionally L. acidophilus LA-5 has shown to inhibit growth of breast cancer cells, and positive effects on chemotherapy patients. An improvement of lipid metabolism has also been linked to L. acidophilus LA-5.

Bifidobacterium is a genus of Gram-positive, non-motile, often branched anaerobic bacteria. They are ubiquitous, endosymbiotic inhabitants of the gastrointestinal tract, vagina and mouth (B. dentium) of mammals, including humans. Bifidobacteria are one of the major genera of bacteria that make up the colon flora in mammals. Some bifidobacteria are used as probiotics. The genus Bifidobacterium possesses a unique fructose-6-phosphate phosphoketolase pathway employed to ferment carbohydrates.Much metabolic research on bifidobacteria has focused on oligosaccharide metabolism as these carbohydrates are available in their otherwise nutrient-limited habitats. Interestingly, infant-associated bifidobacterial phylotypes appear to have evolved the ability to ferment milk oligosaccharides, whereas adult-associated species utilize plant oligosaccharides, consistent with what they encounter in their respective environments (31). As breast-fed infants often harbor bifidobacteria dominated gut consortia, there have been numerous applications to mimic the bifidogenic properties of milk oligosaccharides. These are broadly classified as plant-derived fructo-oligosaccharides or dairy-derived galacto-oligosaccharides, which are differentially metabolized and distinct from milk oligosaccharide catabolism.

Bifidobacterium longum is a species of Gram-positive anaerobic branched rod-shaped bacterium which can be found in the intestines of infant humans. They inhibit the growth of Gram-negative bacteria by producing lactic acid, and digest the complex sugars in human breast milk.

OBJECTIVES

Tuberclosis is the infection disease with bacterial paternity that can affect different part of the body such as respiratory system, derm and digestive system.Isonaizid is a important drug in first line of tuberclusis treating that creates more liver injuries and lactobacillus acidophilus with Bifidobacterium have the prepairing effects in liver so the aim of this study was investigation the effect of Lactobacillus

acidophilus and bifidobacterium at liver tissue injuries that are created by Isoniazid.

MATERIAL AND METHODS

3-1. Designing Method

This study was clinical managing in rat.60 adult male Sprague – dally at the age of 6-7 months and the average weight of 220±60 gram, were ministrated from veterinary faculty of Tabriz Islamic Azad University. These rats were conservated at standard controlled conditions tray of the last scientific and basal recommendation that have been printed by australlian researches (32).All rats have free accessibility to beverage water and specific foods to laboratory animals. These rats were conservated in polycarbonate cages at the gregarious disposition and in standard tempreture that has been defined for rats (18 -26 °c) with 30-70 percent dampness. For retinuing the tempreture, gampness and suitable ventilation, was used from air condinator, thermometer and digital psychrometer with capability of changes recording at the 24 hours period. For accumulation the urine and faeces of these triables, the trepan parings were used.

In this study 60 rats in 4 groups with 15 rats per each group were considered. One of groups was without any injection and with physiological diatery. The name of this group was non treated with NT abbreviation phrase. One another group was Isoniazid that were injected Isoniazid with dose of 50 mg/kg intra peritoneally (IP) with I-1,I-2 and I-3 abbreviation phrases. The third group was probiotics with Isoniazid that received Lactobacillus acidophilus in concentration of 2×10^{9} CFU/ml, Bifidobacterium in concentration of 1×10^{8} CFU/ml with ILB-1,ILB-2 and ILB-3 abbreviation phrases and Isoniazid with dose of 50 mg/kg.The last group was probiotics only receiving with LB-1,LB-2 and LB-3 abbreviation phrases.In each groups at the end of firs,second and third week,the blood was taken after anesthesia.

The liver tissues were sampled after passing of above time and section providing was stained by Hematoxyline and Eosin (H&E) and histological changes were evaluated by using high microscope, NIKON ECLIPSE E200. The degree of liver tissue injuries were graded as follows: 0, absent; 1, minimal; 2, mild; 3,modest and 4,sever. The Kruskal Wallis test was used for determining the significancy of liver injuries.

3-2. Prepairing Lactobacillus acidophilus

In this study Sodium coloride solution (Nacl) 0/9 % was added to Lactobacillus acidophilus KFRI342 lineage and after centrifuge with 5000 round per minute (rpm) for 10 minutes at 4 °c , was altered to lyophilized pouder. For using of this pouder, the solution of Nacl 0/9% was

added to ½ gram of that pouder and finally Lactobacillus acidophilus concentration reached to 2×10^9 CFU/ml (33).

3-3. Prepairing Bifidobacterium

In this study from Bifidobacterium Longum lineage with 4×10^{10}CFU/g pouder shape in the package was used.These package were detained in -20°c and after attenuation by deionized water,it was used. The last concentration of Bifidobacterium in this solution was 1×10^8 CFU/ml (34).

3-4. Biochemical experiments

Blood samples, instantly were placed in centrifuge with 3000 rpm for 10 minutes and the serumic samples were detachmented and were freezed in -80 °c. For biochemical markers measurement such as ALT,AST, direct and total billirubin, ALP, CPK, SOD, MDA, GPX, Albomin ,total protein, CBC and HCT, these serumic and blood samples were carriered to Laboratory of veterinary faculty of Islamic Azad university. The specific laboratory kits (Pars Azmoon, Iran) were used to measurement of these markers.

3-5. Histological and pathological experiments

The liver tissue samples were fixed in formaldehyde 10% for 24 hours (Automatic tissue processor method, EMP-5160).The 5 µm sections were prepaired after water extraction by ethanol from liver tissues. and these sections were colourationed by the method of H&E then these section providing were evaluated by using high microscope, NIKON ECLIPSE E200.

3-6. Liver Enzymatic deliberation

The liver samples were washed by cold NaCl immediately (Ohkama et al, Method) (35) and were placed in specific tissue maintaining and were transferred into phosphate buffer (PH=7/4) then these tissues were haemogenized in Potassium coloride (KCL) at the 0/14 mol/lit concentration. after haemogenization, these samples were centrifuged in 5000 rpm for 20 minutes at 4 °c (36). The product was maintained in -20°c and finally transferred to laboratory of Tabriz Islamic azad University. GPX, SOD and MDA were evaluated in auto analyzer (ALCY on 300) by IFCC-2011 method and the specific laboratory kits (Pars azmoon-Iran).

3-7. Data analyses

All data were analysed by one way analyses of varience (ANOVA) followed by Dunnett´s test using statistical package for social sciences (SPSS) version 18. $P<0/05$ was considered significant.

RESULTS

In this study the values of ALT and AST showed a significant increasing at first (p=0/04), second (p=0/03) and third week (p=0/02) compared with control group. Lactobacillus acidophilus with

Bifidobacterium consumption, significantly decreased the values of ALT and AST compared with control group at first (p=0/01), second (p=0/04) and third week (p=0/00/03). These probiotics with Isoniazid consumption significantly decreased the values of ALT and AST compared with Isoniazid only consumption at first (p=0/01), second (p=0/02) and third week (p=0/02). These parameters had not significantly changes in probiotics consumers at first week (p=0/08) but had significant decreasing at second (p=0/03) and third week (p=0/04).

Lactobacillus acidophilus with isoniazid significantly decreased the values of direct and indirect billirubin compared with Isoniazid only consumption at first (p=0/03), second (p=0/03) and third week (p=0/04). Isoniazid significantly increased these parameters at first (p=0/03), second (p=0/02) and third week (p=0/04). Probiotics significantly decreased these values at second (p=0/03) and third week (p=0/02) but not significantly at first week (p=0/09). The values of direct and indirect billirubin in Lactobacillus acidophilus and Bifidobacterium consumption groups were decreased compared with probiotics and isoniazid consumption at first week but not significantly (p=0/08) and significantly at second (p=0/04) and third week (p=0/03). Lactobacillus acidophilus and bifidobacterium significantly decreased the values of total billirubin at first (p=0/03), second (p=0/03) and third week (p=0/04) compared with Isoniazid only consumption. Isoniazid was leaded to significant increasing of these parameters at first (p=0/03), second (p=0/03) and third week (p=0/04) compared with control gpoup. Probiotics only consumption created insignificant changes at second week (p=0/09) and significant changes at first (p=0/04) and third week (p=0/03) compared with control group.Lactobacillus acidophilus and Bifidobacterium with Isoniazid significantly decreased the values of total billirubin at first (p=0/04), second (p=0/02) and third week (p=0/03) compared to Isoniazid only consumption.

The serumic values of Albomin and total protein significantly were decreased after Isoniazid consumption at first (p=0/04), second (p=0/03) and third week (p=0/01) compared with control group. Isoniazid with lactobacillus acidophilus and Bifidobacterium could not decrease these parameters at second week (p=0/09).It´s decreasing only at third week was significant (p=0/03).lactobacillus acidophilus and Bifidobacterium without isoniazid significantly decreased these parameters at first (p=0/04), second (p=0/03) and third week (p=0/02) compared with control group. The values of

liver SOD, GPX and total protein significantly were decreased after Isoniazid consumption at first (p=0/03), second(p=0/04 and third week (p=0/02) compared with control group. Isoniazid with lactobacillus acidophilus and Bifidobacterium or only these probiotics consumption significantly increased the values of total protein at first (p=0/04), second(p=0/04) and third week (p=0/03) compared to Isoniazid only consumption group.

Isoniazid with probiotics significantly increased the values of GPX at second (p=0/02) and third week (p=0/03).

The values of CPK were increased after Isoniazid consumption at first (p=0/03), second (p=0/04) and third week (p=0/01) compared with control group but Lactobacillus acodophilus and Bifidobacterium with Isoniazid significantly decreased these values at first(p=0/03), second (p=0/04) and third week (p=0/03) compared with only Isoniazid consumption. probiotics significantly decreased the values of CPK at second (p=0/02) and third week (p=0/02) but not significantly decreased the values of CPK at second (p=0/02) and third week (p=0/02) but not significantly at first week (p=0/07). The values of CPK were decreased after probiotics consumption at first (p=0/04), second (p=0/03) and third week (p=0/02) significantly compared with consumption of these probiotics with Isoniazid. The number of Eosinophil cells were increased at second (p=0/04) and third week (p=0/04) compared with control group. Lactobacillus acidophilus and Bifidobacterium significantly decreased these values at third week (p=0/02) compared with Isoniazid consumption group.All results off this study were shown in table 1-2 and figure1.

Table 1. The values of ALT, AST, Direct and total billirubin, Albumin in liver of rats in all groups

Parameter	AST	ALT	Direct billirubin	Total billirubin	Albumin
Group					
NT-1	118.57±0.37	74.20±0.91	0.92±0.07	0.57±0.06	1.56±0.11
NT-2	120.04±0.53	76.37±0.21	0.81±0.05	0.51±0.04	1.54±0.03
NT-3	120.08±0.70	75.82±0.56	0.80±0.57	0.49±0.04	1.51±0.39
I-1	160.57±0.56	200.36±0.19	1.44±0.18	0.71±0.05	0.82±0.31
I-2	161.43±0.53	202.31±0.21	1.70±0.10	0.78±0.47	0.79±0.35
I-3	161.06±0.69	204.33±0.09	2.60±0.14	0.79±0.61	1.05±0.02
ILB-1	100.77±0.44	153.91±0.47	1.16±0.04	0.48±0.59	1.65±0.03
ILB-2	100.63±0.47	146.31±0.16	1.10±0.04	0.38±0.47	1.47±0.04
ILB-3	90.30±0.19	152.37±0.19	0.94±0.21	0.24±0.02	1.39±0.03
LB-1	93.57±0.78	50.57±0.56	0.53±0.05	0.40±0.15	1.36±0.07
LB-2	87.31±1.01	43.39±0.74	0.46±0.03	0.38±0.01	1.45±0.11
LB-3	83.00±1.00	33.15±0.65	0.35±0.04	0.35±0.04	1.74±0.14

Table 2. The values of Total protein,MDA,SOD,GPX,CPK and ALP in liver of rats

Paramer Group	Total protein	MDA	SOD	GPX	CPK	ALP
NT-1	49.23±0.56	1.33±0.48	4.26±0.13	389.23±1.17	206.17±0.35	175.23±5.23
NT-2	51.03±0.56	1.35±0.45	4.16±0.04	390.75±0.54	205.76±0.62	172.94±1.83
NT-3	50.79±0.48	1.26±0.04	4.18±0.06	389.84±0.53	206.71±0.40	172.93±1.72
I-1	48.83±0.58	1.32±0.03	3.85±0.13	372.75±0.48	207.68±0.43	1322.33±4.55
I-2	43.40±0.15	1.42±0.04	3.14±0.59	368.11±0.44	209.02±0.50	1475.92±3.30
I-3	40.72±0.51	1.49±0.04	1.99±0.35	365.00±0.44	210.24±0.86	1494.37±2.82
ILB-1	51.75±0.62	1.34±0.03	4.36±0.93	395.86±0.59	207.08±0.62	1415.57±5.29
ILB-2	50.82±0.69	1.30±0.02	4.68±0.10	408.67±1.54	205.75±0.54	1305.00±4.43
ILB-3	53.15±0.43	1.22±0.03	5.12±0.19	553.17±3.13	204.68±0.51	1255.00±3.00
LB-1	63.57±0.00	1.43±0.17	20.05±0.02	445.00±1.07	206.21±0.43	171.57±1.13
LB-2	68.49±0.95	1.36±0.12	21.31±0.05	446.74±0.32	198.78±0.15	175.13±3.14
LB-3	75.40±0.0	1.15±0.14	24.65±0.04	449.40±0.40	198.61±0.73	177.00±6.00

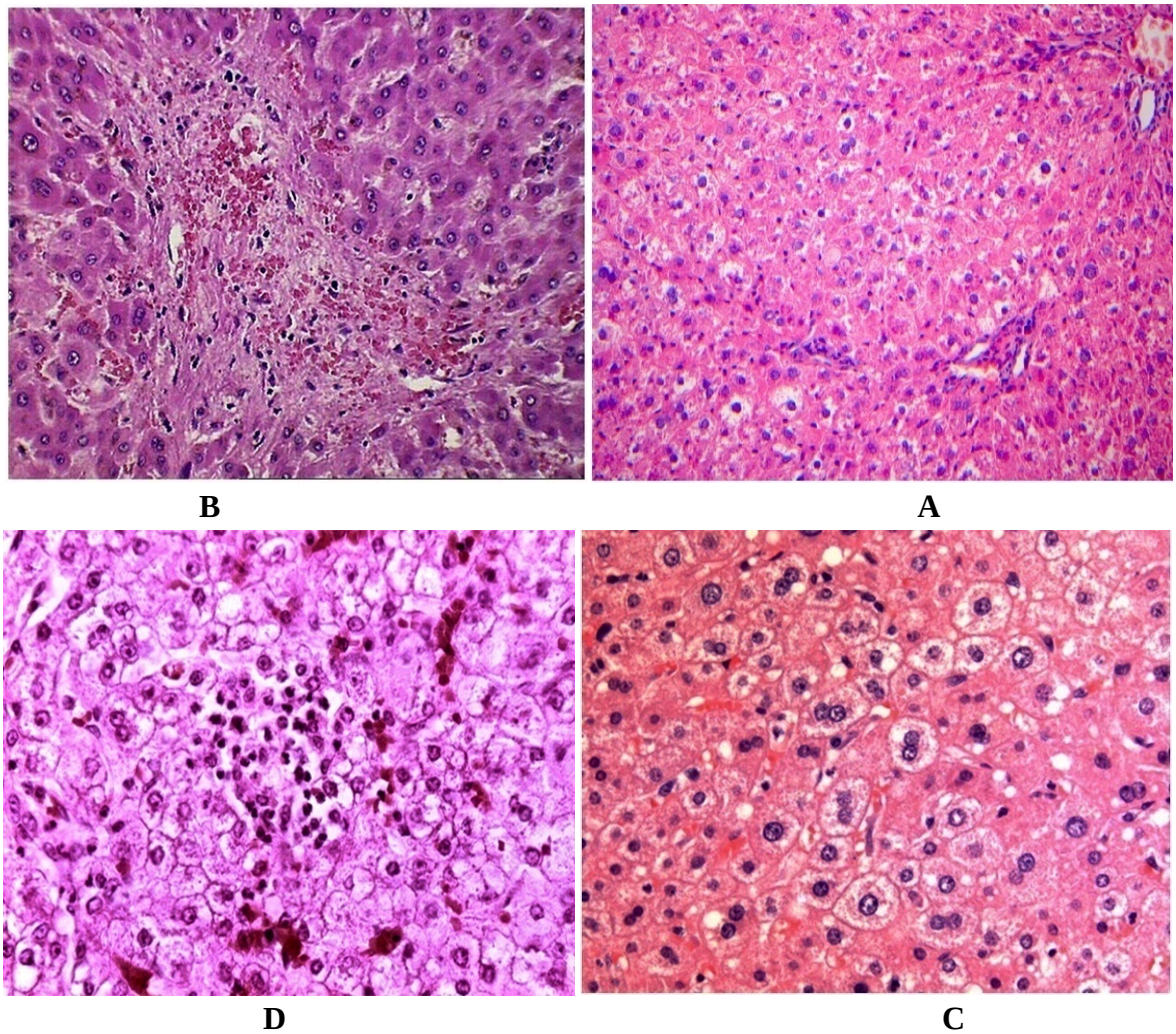

Figure1. A: The microscopic view of liver tissue in NT group. The Histological structure of liver tissue is normal (H&E ×40). B:The microscopic view of liver tissue in Isoniazid group was received Isoniazid 50mg/kg 3 week. Wide destination of inter hepatocytes space, RBC infiltration, Generalized hydropic degeneration and WBC infiltration are absorved (H&E ×40). C: The microscopic view of liver tissue in Isoniazid with lactobacillus acidophilus and Bifidobacterium treated group for 3 week only local hyperemia and local hydropic degeneration are absorved (H&E ×40). D: The microscopic view of liver tissue of rat belonged to Lactobacillus acidophilus and Bifidobacterium for 3 week. Hyperemia, local inflammation and a fiddling local hydropic degeneration are seen.

DISCUSSION

Probiotics specially Lactobacillus acidophilus and Bifidobacterium that were studied are live microorganisms that are used for prepairing the digestive system deficiencies such as diarrhea,vomiting,constipation,lactose intolerance and prevention of proliferation the bowel pathogenic bacteria. In more investigations that were done with base of the Lactobacillus acidophilus and Bifidobacterium in liver, these probiotics are leaded to decreasing the Ammoniac in portal system by innate Urease inhibiting properties and basically decreasing all Urease activity that are leaded to ammoniac increasing in hepatic portal system. These activities significantly decrease the Ammoniac penetration in bowel. Increasing the Ammoniac in blood, accured in such some hepatic diseases, hepatic deficiencies and poisoning by Urea that are leaded to brain and nervous disproportions. Probiotics inhibit from absorption of poisons by ammoniac absorption decreasing that these conditions decrease inflammation and oxidative stress. Decreasing the oxidative stress prevents and treates the hepatic diseases. Isoniazid is used for first line of treating the tuberclusis disease. The mechanism of this drug is prevention the adolescence of tuberclusis bassilles and creating the Micolic acid that is used for bacterial cell wall. Isoniazid can enter into macrophage cells and finally affects intra and extra cell organisms. These effects are creating by changing the microzomal enzymes that are interference in drug metabolism in liver. The mentioned enzymes are in communication with liver cytochrome P-450. In the study that Chen and other cohorts have done, Isoniazid 48-72 hours after using was leaded to beginning the inflammation and liver injuries. In the study that Nadia Osman and cohorts have done, Lactobacillus acidophilus and Bifidobacterium shown their protective effects after first week. In this study the effect of Isoniazid, Lactobacillus acidophilus and Bifidobacterium in liver at first, second and third week was checked. For reaching to this target, the liver histomorphometric changes, biochemical and inflammatory markers such as: ALT, AST, SOD, CPK, MDA and GPX, were studied. ALT and AST for liver injuries checking, CPK and ALP for liver inflammatory checking, SOD, GPX and MDA for oxidation of hepatocytes checking , were studied (37).

In this study the values of ALT and AST were increased significantly compared with control group that it is because of hepatic load increasing, cellular damages and metabolically changes. The increasing of hepatic loading is leaded to decreasing the

liver bioavailability and function. Decreasing the liver function prevents from the main liver operation that is detoxification. Furthermore the different substances are aggregation and create the hepatocytes damages (38). One symbol of liver damages is releasing the ALT and AST enzymes in surgly formation while Lactobacillus acidophilus and Bifidobacterium with Isoniazid or Lactobacillus acidophilus and Bifidobacterium only consumption, decreased the values of ALT and AST compared with control group like the Salim Abobaker and cohorts studies (39). This mechanism is the resulting from prevention of hepatic damages by Lactobacillus acidophilus and Bifidobacterium. These probiotics regenerate the creatness damages resulting from Isoniazid, and decrease the values of ALT and AST that are the hepatocytes damages.

The values of direct and indirect billirubin were increased after Isoniazid consumption compared with control group. This is because of the liver injuries resulting from Isoniazid. In this condition picking up, conjucation and secretion are disproportioned. Billirubin is the reductioned combination that is synthesized in reduction processing period, by Billiverdine reductase enzyme that is synthesized from Billiverdine. This yellow and reductioned combination probably is conjucated or nonconjucated. In conjucated condition, it is renamed indirect billirubin and in nonconjucated condition, it is renamed direct Billirubin. The objective of direct billirubin is that this combination can be entered directly into phosphorylation-reduction rebound (40).

The main place of conjucation and deconjucation is liver that this function is the responcibility of hepatocytes. The liver damages that resulting from Isoniazid, are leaded to disproportions in liver function. Furthermore the values of conjucated and nonconjucated Billirubin are increased and these combinations aggregations in liver because of liver tribulationed secretial function. Finally these combinations aggregations in liver is causing positive feedback of increasing the conjucated and nonconjucated Billirubin. Furthermore it is concluded that the liver damages resulting from Isoniazid, causing the agonistically injuries in hepatocytes by increasing the Billirubin .So at long periods we will be voucher for increasing these combinations compared with short periods of time. In this study Lactobacillus acidophilus and Bifidobacterium significantly decreased the values of total Billirubin compared with control group that this is resulting from the compensation affect of these probiotics at liver injuries. These probiotics prepair the

liver function by the effect of hepatocytes. Furthermore the conjucation and secretion procedure that had been preturbationed, returns to normal condition and the values of total Billirubin decrease and reach to normal manner.

The serumic values of Albumin and total protein were decreased significantly compared with control group that is resulting from metabolically changes and renal secretion of these parameters. Isoniazid with Lactobacillus acidophilus and Bifidobacterium cannot decrease these values because these probiotics prevent the tribulation effects of isoniazid at kidney and metabolism. Albumin is protein that is used to transmitting the substances. This protein is the vital requirements to body. Furthere more at the physiological conditions, albumin is not excretioned from kidney and even abit excretion of this protein in kidney shows the basically tribulations of kidney. Isoniazid changes the capillary penetration by effect the renal glumerolares. So the membrane of glumeroles are penetrated while at physiological conditions they were not penetrated and this manner is leaded to Albumin excretioning. Decreasing of Albumin causes decreasing the serumic protein directly. So Isoniazid decreases Albumin and serumic total protein. Lactobacillus acidophilus and Bifidobacterium decrease the values of Albumin and total protein that is resulting from cellular metabolism increasing specially hepatocytes metabolism. These probiotics increase the hepatocytes function so increase production of these proteins. The metabolism is not only in this case. (40, 41)

The important part of cellular metabolism consists of gluconeogenesis that it is shown in cronic starvations. Gluconeogenesis produces glucose from different combinations such as amino acids. Furthermore increased metabolism that is resulted from Lactobacillus acidophilus and Bifidobacterium consumption, increases gluconeogenesis and Albumin to glucose changing. These conditions decrease the blood Albumin. So a significant contact is existed among hepatocytes function, Albumin produced increasing by cellular metabolism increasing and decreasing the Albumin in blood. The cellular metabolism processing is a continuously processing in the body that exists even without probiotics using while increasing the hepatocytes function in this study only is created by using these probiotics so the cellular metabolism is more important to the body physiologically and glucose producing is more important than Albumin synthesis (42). These conditions decrease the values of Albumin.

The values of SOD, GPX and liver total protein were decreased after Isoniazid consumption. Isoniazid has two important destroying role in liver. The first role is regenerative liver damages that is created by the chemical nature of this drug and another destroying role of this drug that is excessive and non regenerative, that is created at long period consumption of this drug (43).

Isoniazid creates liver damages at long period of time and more damages are accured at hepatocytes membrane. The membrane lesions can create oxidative and radical combinations that are dangerous and harmful for the cells. The last combination that is produced by hepatocytes membrane damages, is hydrogen peroxide that causes perturbations in other cell organells after entering into the cells. Oxidative combinations are leading the cellular apologia barrier that this response shows itself typically by producing the combinations against the free radicals that those names are antioxidant.

The important antioxidants are SOD and GPX. So these combinations are expenses because of increasing the damages and oxidative substances. Furthermore the values of SOD and GPX are decreased. Isoniazid with Lactobacillus acidophilus and Bifidobacterium or Lactobacillus acidophilus and Bifidobacterium without Isoniazid increased the values of GPX and total protein but decreased the values of MDA. In the study of Zang and Wang (44) these results were shown too because of free radicals attack to the combinations that can take them electrons such as unsaturated fatty acids so the values of MDA are increased and GPX is expensed for destroying these substances.

Increasing MDA, creates the oxidative stress. SOD and GPX are the antioxidants that are against the free radicals. Lactobacillus acidophilus and Bifidobacteriumcause the arrangement and solidarity of the body antioxidant specially GPX on the other hand these antioxidants are expenses to contrst with free radicals and oxidative combinations. Lactobacillus acidophilus and Bifidobacterium cause the endurance and prevention of expressly destroying of antioxidant combinations specially GPX so the apologia potency and antioxidant defensive renitency are increased in front of oxidative combinations but the lone exception in this case is SOD (45). Lactobacillus acidophilus and Bifidobacteriumcan not increase the defiance of attendance of the SOD. Furthermore after Isoniazid consumption the oxidative stress is increased. In this study the values of CPK in liver, significantly are increased compared with

control group because of increasing the toxicity of the liver that is resulted from isoniazid consumption.

Increasing the ALT, AST and decreasing the SOD and GPX shows the sever liver damages. The values of MDA in liver significantly decreased after Isoniazid consumption while Lactobacillus acidophilus and Bifidobacterium increased these values.

Liver is the important organ of the body for metabolism and bioavailability. This organ is more sensitive for oxidative damages on the other hand exported suitable response for inappreciable damages so the values of enzymes specially oxidant and antioxidant combinations are changed in liver tribulations and when the liver antioxidant such as SOD and GPX are decreased, the another part of the liver replaces these (46). Furthermore the decreasing of these antioxidant combinations wouldn't be susceptible for measurement. Isoniazid consumption decreases the values of SOD and GPX in liver but these antioxidants immediately are replaced and this replacement is more than first condition.

Liver is created by more specialist cells for secretional activities. These cells are placed in cellular group formation around the central duct. This duct depletes the bile secretions that are produced by hepatocytes. These ducts meet together and create the liver internal bile ducts. Liver divided to two parts that is named two lobe, that are right and left lobes. The bile ducts after out coming from the lobe, are named idem lobe name. These external liver ducts meet together and evacute to bile bladder (47).

In this study consumption of Isoniazid created the acute damages in liver that these injuries were observed such as blood cells infiltration in injured regions.

Histamin is the important substance at inflammatory response that cause vessels widing inflammatory processing exacerbation (48). Hyperemia and aggregation of red blood cells show the acute injuries and liver tissue inflammatory. Isoniazid increases the releasing of combinations of oxidative stress such as hydrogen peroxide in cell membrane. These oxidative combinations are entering into cytoplasmic enclosure. The elementary response for these damages is the secretion of vessels dilator combinations such as Histamine, Bradykinine, and Trombine. Histamin increases the blood coming to injured regions by vasodilatation and this is causing the existence of inflammatory cells in injured regions that it is creating inflammation. At long period of time specially at second and third week, inter hepatocytes space is widing because of continuous contact of hepatocytes with

damages. These conditions cause the bodling inter stitial fluid. Changing the cytoplasmic content, dropsical formation at elementary process of inflammation and finally creating the hydropic degeneration are seen. The pathological injuries in liver are because of the increasing of hepatic load (49).

Liver has two important activity by attention to producing and secretion the different proteins that are pre loading and post loadind. Pre loading are the combinations that are entering into liver to bioavailability and post loading are the liver secretions. Isoniazid is a drug that should pass from these hepatic canal to detoxification. Long period of time consumption of this drug causes the saturation of this canal so hepatocytes are became more active that this is caused different cellular damages.

Isoniazid remaining that are not entered to bioavailability, increases the hepatocetes pressure. Hepatocytes injuries that create damagefull combinations such as free radicals and increasing the pressure of the hepatocytes are increasing the inter hepatocytes space. The inflammation existes in injured regions to physiologically responsing. The inflammation cells attach to these forspent spaces. Increasing ALT, AST, MDA and decreasing GPX and SOD in liver are supporting from these injuries creating. Lactobacillus acidophilus and Bifidobacterium are significantly decreasing these injuries specially at second and third weeks after consumption by effect the hepatic bioavailability. Decreasing ALT, AST and MDA and increasing GPX and SOD in liver are the confirmatory responsing from decreasing of these injuries. Lactobacillus acidophilus and Bifidobacterium are the probiotics and powerful antioxidants that significantly decreasing the free radicals that were produced by cell membrane injuries (50).

CONCLUSION

Tuberclusis is the infection disease that has worldwide expanding and in sensitive regions it can be endemically.

Military forces are attemping contrast with this disease in these regions and this conditions leading to increasing the risk of this disease. Isoniazid has the damagefull effects in liver and lactobacillus acidophilus with Bifidobacterium have the prepairing effects in liver. From these results we conclude that these probiotics significantly decrease the damagefull effects of Isoniazid in liver tissue. The results of this study are usefull at the future researches in human cases and finally in military forces.

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Authors´ contribution

Behrouz Mikailpour drafted the manuscript, abstracted data, submitted the article and guarantor. Ramin Hamidi developed the original idea and the protocol. Nader Markazi Moghaddam analyzed data, helped in writing the article and collected the data. Soheil Ehsan Alavi explanates the histological and pathological changes of tissues.

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REFERENCES

[1] Karthikeyan S. Hepatotoxicity of isoniazid. A study on the activity of marker enzymes of liver toxicity in serum and liver tissue of rabbits. Indian J Pharmacol 2004; 36:247-9.

[2] Yue J, Peng R, Yang J, Kong R, Liu J. CYP2E1 mediated isoniazid induced hepatotoxicity in rats. Acta pharmacol Sin 2004; 25: 699-704.

[3] Ravinder Pal, Satyavati Rana, Kim Vaiphei, Kartar Singh. Effect of different doses of carotenoids inisoniazid-rifampicin induced hepatotoxicity in rats. Tropical Gastroenterology 2008;29: 3:153–9

[4] Shadi Sar Kheyr Kheyabany , Fatemeh Nabavizadeh,Gholam Hassan Vaezi, Ali Mohammad Alizadeh, Hossein Nahrevanian,Azam Moslehi,et al. Protective Effect of Ghrelin on Isoniazid-induced Liver Injury in Rat. Journal of Stress Physiology & Biochemistry 2013;21:273-82.

[5] Shen C, Meng Q, Zhang G and Hu W. Rifampicin exacerbates isoniazid-induced toxicity in human but not in rat hepatocytes in tissue-like cultures. British Journal of Pharmacology 2008; 153: 784–91.

[6] Arshad Ali Noorani, Navdeep Saini, Koyal Saini , Kale MK. Hepatoprotective Effect of Rimonabant Against Isoniazid Induced Liver Damage In Albino Wistar Rats. International Journal of Pharmaceutical & Biological Archives 2010; 1:473 –7.

[7] Sude Eminzade, Fikriye Uras ,Fikret V Izzettin. Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. Nutrition & Metabolism 2008; 5:18

[8] Yong Lian,[#1,2] Jing Zhao,[#1] Peiyu Xu,[2] Yimei Wang,[1,*] Jun Zhao,[1] Li Jia et al. Protective Effects of Metallothionein on Isoniazid and Rifampicin-Induced Hepatotoxicity in Mice, 2013; 8(8): e72058, Published online 2013 August 13. doi: 10.1371/journal.pone.0072058, PMCID: PMC3742471

[9] S. Angala Parameswari[I,*]; T. S. Mohamed Saleem[II]; K. B. Chandrasekar[III]; C. Madhusudhana Chetty. Protective role of Ficus benghalensis against isoniazid-rifampicin induced oxidative liver injury in rat. Rev. bras. farmacogn. vol.22 no.3 Curitiba Ma y/June 2012 Epub Jan 17, 2012

[10] Ravinder Pal, Kim Vaiphei, Arbab Sikander, Kartar Singh, Satya V Rana. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. 1007-9327 CN 14-1219/R, World J Gastroenterol, 2006 January 28;12(4):636-639.

[11] Ravinder Pal, Satyavati Rana, Kim Vaiphei, Kartar Singh. Effect of different doses of carotenoids in isoniazid-rifampicin induced hepatotoxicity in rats.tropical gastro entrology.

[12] Sudha, A.; srinivasan, P.; manikandaselvi, S.; thinagarbabu, R. Pub. Date. Protective effect and antioxidant role of achyranthus aspera l. Against ethanol-induced oxidative stress in rats. International Journal of Pharmacy & Pharmaceutical Sciences; Apr2012 Supplement 3, Vol. 4, p280

[13] Heidari R,Babaei H ,Eghbal MA. Ameliorative effects of taurine against methimazole-induced cytotoxicity in isolated rat hepatocytes. Scientia pharmaceutica 80:4 2012 Dec pg 987-99

[14] Georgieva N, Gadjeva V, Tolekova A, Dimitrov D. Hepatoprotective effect of isonicotinoylhydrazone SH7 against chronic isoniazid toxicity. Die Pharmazie 60:2 2005 Feb pg 138-41

[15] Seema Sharma[1] / Professor Suresh Vir Singh Rana. Melatonin Improves Liver Function in Benzene-Treated Rats. Archives of Industrial Hygiene and Toxicology. Volume 64, Issue 2, Pages 219–227, ISSN (Print

[16] A.H.M. Viswanatha Swamy, Rucha V. Kulkarni, A.H.M. Thippeswamy, B.C. Koti, and Aparna Gore. Evaluation of

hepatoprotective activity of Cissus quadrangularis stem extract against isoniazid-induced liver damage in rats. Indian J Pharmacol. 2010 December; 42(6): 397–400.

[17] Jürgen Schrezenmeir and Michael de Vrese. Probiotics, prebiotics, and synbiotics—approaching a definition.Am J Clin Nutr 2001;73:361-4.

[18] Bucher HC, Griffith LE, Guyatt GH, Sundre P, Naef M, Sendi P, et al. Isoniazid prophylaxis for tuberculosis in HIV infection: a meta-analysis of randomized controlled trials. AIDS 1999; 13:501-7.

[19] Lilly D M,Stillwell H. Probiotics: Growth-promoting factors produced by microorganisms. Science New York, N.Y 1965:147 :747–8.

[20] Parker R B.Probiotics, the other half of the antibiotic story. Animal Nutrition and Health 1974; 29: 4–8.

[21] Mach T. Clinical usefulness of probiotics against chronic inflammatory bowel diseases. Journal of Physiology and Pharmacology 2006;9: 23–33.

[22] Cremonini F, Di Caro S, Nista EC. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Aliment. Pharmacol 2002; 16 :1461–7.

[23] Zhang Y, Du R, Wang L, Zhang H. The antioxidative effects of probiotic Lactobacillus casei Zhang on the hyperlipidemic rats. European Food Research and Technology 2010;231:151-8

[24] Devlin TM. Text book of Biochemistry with clinical correlations. 6nded.John wiley and sons; 2006.P.134-9

[25] Baati L L, Fabre-Gea C, Auriol D, Blanc P J. Study of the cryotolerance of Lactobacillus acidophilus: Effect of culture and freezing conditions on the viability and cellular protein levels. International Journal of Food Microbiology 2000; 59: 241–7

[26] Anderson J, Gilliland S. Effect of fermented milk (yogurt) containing Lactobacillus acidophilus L1 on serum cholesterol in hypercholesterolemic humans. J Am Coll Nutr 1999;18: 43–50.

[27] Forsum U, Holst E, Larsson P, Vasquez A, Jakobsson T, Mattsby-

Baltzer I. Bacterial vaginosis—a microbiological and immunological enigma. APMIS 2005; 113: 81–90.

[28] Ljungh A, Wadstrom T. Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol 2006;7: 73–89

[29] Sanders ME, Klaenhammer TR. Invited review: the scientific basis of Lactobacillus acidophilus NCFM functionality as a probiotic. J Dairy Sci 2001; 84: 319–31

[30] De Roos N, Katan M. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr 2000;71: 405–11.

[31] Akihito E , Okada S, Morita HG.Molecular profiling of Lactobacillus, Streptococcus, and Bifidobacterium species in feces of active racehorses. Gen Appl Microbiol 2007; 53:191-200

[32] Gerald W. Identification of Lactobacilli and Bifidobacteria. Current Issues Molec. Biol 1999; 1: 53-64

[33] Chanter N. The molecular basis of Streptococcus equi infection and disease. Microbes Infect 2002; 4 :501–10

[34] Goreet RH, Grisham CM. Biochemistry.3nd ed. Thomson,Brooks;2005 .P.281-8

[35] Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H .Trends in oxidative aging theories. Free Radic. Biol. Med 2007;43: 477–503.

[36] Ran Q, Liang H, Ikeno Y. Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. J. Gerontol. A Biol. Sci. Med. Sci 2007 ;62 :932–42.

[37] Muller F L, Lustgarten M S, Jang Y, Richardson A,Van Remmen H. Trends in oxidative aging theories. Free Radic. Biol. Med 2007; 43: 477-503

[38] Kevin C. Kregel, David L. Allen, Frank W. Booth, Monika R. Fleshner, Erik J. Henriksen et al.The APS Resource Book for the Design of Animal Exercise, Protocols. American Physiological Society, 2006;100-132

[39] Hobart, Tasmania .Australian Antarctic Division on behalf of the Antarctic Animal Care and Ionising Radiation Usage Ethics Committee. Guidelines for

research involving animal experimentation or use of ionizing radiation. Commonwealth of Australia 1989;33:54-8

[40] Fried MW, Navarro VJ, Afdhal N, Belle SH, Wahed AS et al. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. National Center for Biotechnology Information2012; 18:274-82.

[41] Jin-Hee Chang, Youn Young Shim, Seong-Kwan Cha, Martin J. T. Reaney and Kew Mahn Chee Effect of Lactobacillus acidophilus KFRI342 on the development of chemically induced precancerous growths in the rat colon . Journal of Medical Microbiology. 2011;11:130-9

[42] Rowland I.R, Rumney C.J, Coutts J.T and Lievense L.C. Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis 1998;30:281–5.

[43] Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem 1979; 95: 351-8.

[44] Cottrell E. C, Cripps R. L, Duncan J. S, Barrett P, Mercer J. G, Herwig A, and Ozanne S. E. Developmental changes in hypothalamic leptin receptor: relationship with the postnatal leptin surge and energy balance neuropeptides in the postnatal rat. Am J Physiol Regul Integr Comp Physiol 2009; 296:631–9.

[45] Baydas G, Canatan H, Turkoglu A. Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus, J. Pineal Res 2002; 32:225–30.

[46] Feng-Ching Hsieh, Chia-Lin Lee, Chee-Yin Chai, Wan-Tzu Chen, Ying-Chen Lu et al.Oral administration of Lactobacillus reuteri GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. Nutrition & Metabolism 2013; 10:35

[47] Renato Sousa, Jaroslava Halper, Jian Zhang, Stephen J , Wan-I O .Effect of Lactobacillus acidophilus supernatants on body weight and leptin expression in

rats.. BMC Complementary and Alternative Medicine 2008; 8:5.

[48] Abou-Baker Salim, Abou-Arab A.A.K, Azza Zohair. Antitoxin effect of Streptococcus thermophilus and Lactobacillus delbrekii ss. bulgaricus against benzo[a]pyrene in rats. Journal of Applied Sciences Research 2012;8: 3866-73.

[49] Guven A, Yavuz O, Cam M, Ercan F, Bukan N, Comunoglu C, Gokce F. Effects of melatonin on streptozotocin-induced diabetic liver injury in rats. Acta histochemica 2006;108: 85-93.

[50] DIYA ADAWI F. Effect of Lactobacillus Supplementation with and Without Arginine on Liver Damage and Bacterial Translocation in an Acute Liver Injury Model in the Rat. HEPATOLOGY 1997;25:642-7.