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Biological evaluation of synbiotic fermented milk against lead acetate contamination in rats

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Abstract

The present study investigated some biological effects of synbiotic fermented milk in rats receiving lead acetate in drinking water. Synbiotic fermented milk was prepared by mixing probiotic fermented milk with honey, garlic, ginseng, cod liver oil and chicory inulin. Glutathione-S-transferase (GST), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated in rats receiving lead acetate alone and in rats receiving lead acetate and synbiotic milk, in addition to the negative control group. The obtained results showed a significant increase in the activities of ALT and AST in sera of rats receiving lead acetate compared with the negative control. The activities of ALT and AST increased from an average of 23.0 to 37.3 and from 160 to 220 IU/L, respectively. The activities of these enzymes in rats receiving lead together with the synbiotic milk were almost similar to the activities of the enzymes of rats fed basal diet (negative control). The mean values of ALT and AST in lead-treated group fed synbiotic fermented milk were 25±3.6 and 156±21.36 IU/L compared with the positive control 37.33±2.51 and 220.66±28.88 IU/L respectively. The obtained results showed that GST activity in sera of rat fed on synbiotic fermented milk was increased with mean value of 23.438 M/min compared with negative and positive controls with mean values of 12.01 and 13.95 M/min respectively. Data imply that synbiotic fermented milk containing honey, garlic, ginseng, cod liver oil and chicory inulin may play a role in protection against lead acetate contamination in rats by increasing the activity of the enzyme GST that requires the antioxidant glutathione as substrate, thus protecting the liver against the oxidative damage.

Key words: Lead, synbiotic fermented milk, glutathione, GST, ALT, AST, rats.

Introduction

Heavy metals are widespread chemical contaminants in the environment of man and animals [1]. Lead is found in all parts of our environment, and the toxic effects caused by lead affect the nervous, gastrointestinal and haemopoietic system [2]. The pathotoxicity of lead is not well understood and various mechanisms have been suggested [3].

Recent studies in various animals have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes and interfering with some essential metals needed for antioxidant enzymes, such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) [4-6]. In addition to these enzymes, glutathione–S-transferase (GST), which provides glutathione (GSH) and helps to neutralize the toxic neutrophiles [7], is also affected by lead. Lead also increases the susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, these factors may be responsible for the toxic effects of lead, and maintaining the cellular antioxidant capacity appears to provide a partial remedy. The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidants may play a role in the treatment of lead poisoning because the conventional treatment has its side effects.

In recent years, complementary medicine has gained increased popularity. Food manipulation and traditional plant therapies are commonly prescribed by ayurvedic and other folk systems of medicine in various countries including Saudi Arabia. Oral consumption of probiotics (live microbial feed supplements that enhance the host health by modulating the intestinal microbial balance) has been associated with the prevention, alleviation or cure of diverse intestinal disorders such as lactose intolerance and viral and bacterial diseases [8]. Considerable evidence has implicated lactobacilli in a number of potentially beneficial roles, *viz.*, immunostimulation, pathogen exclusion, production of bioactive materials, anticarcinogenic activity, deconjugation of bile acids, etc. In the face of growing opportunities for functional foods, dietary adjuncts and health-related products, it is prudent to understand the role of various lactobacilli under *in vivo* conditions. Lactic acid bacteria (LAB) such as *Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum* and *B. longum* have been used as probiotics in humans [9]. They are well-known for their extensive use in the preparation of fermented food products. In addition, the potential health benefits they may exert in humans have been intensively investigated during the last century. However, the mechanisms underlying the health promoting traits attributed to LAB, especially lactobacilli, remain vastly unknown and this has impaired the rational design of probiotic screening methods with accurate predictive value. Probiotics, prebiotics (non-digestible components of food) and synbiotics (combination of pre- and pro-biotic) aimed at improving intestinal health currently represent the largest segment of the functional foods market in Europe, Japan and Australia. Evidence continues to emerge demonstrating that these ingredients have a potential to improve the general health human conditions and improve the LDH/HDL cholesterol ratio [10].

The purpose of the present study is to investigate the efficacy of synbiotic fermented milk in protecting the general health conditions of rats by measuring the activities of some liver enzymes and GSH–dependent enzyme, GST.

Material and Methods

Animals and diets: Male albino Wistar rats (100-120 g) were housed in cages under standardized conditions at temperature of 25±2ºC and relative humidity of 60% and exposure to a 12-h light/ 12-h dark. Rats were fed regular pelleted rat chow diet. The composition of the basal diet used in this study was as follows: milk protein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%) and starch (64 %).

Probiotic fermented milk cultures: Starter cultures of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Lab., Copenhagen, Denmark.

Preparation of probiotic fermented milk: Preparation of probiotic fermented milk was carried out according to the methods of traditional yoghurt manufacturing described by Tamime and Robinson [11]. Bovine milk obtained from Al Qassim University Farm (Saudi Arabia) was standardized to achieve about 12% solid contents, heated at 85°C for 30 min, cooled to 40°C, inoculated with *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* and was then incubated for 4-8 h at 42ºC. After coagulation, the curd was tested for pH, stirred in electric blender and stored at refrigeration temperature of 4-6±2°C. The chemical composition of the probiotic fermented milk is shown in Table 1.

Table 1. Chemical composition of probiotic fermented milk (%).

Total solids	Carbohydrate	Fat	Protein	Ash
12.90	4.66	3.11	2.91	0.90

Preparation of synbiotic fermented milk: Probiotic fermented milk was mixed with 50% honey, 1% garlic, 1% ginseng, 1% cod liver oil, 1% apple acid and 1% chicory inulin. The chemical composition of honey, garlic powder and cod liver oil is shown in Tables 2-4 respectively.

Table 2. Chemical composition of honey (%).

Carbohydrate	Water	Fructose	Glucose	Galactose
82.12	17.10	35.75	35.75	3.10

Table 3. Chemical composition of garlic powder (%).

Carbohydrate	Protein	Fat	Water	Ash
72.71	16.8	0.76	6.45	3.29

Table 4. Chemical composition of cod liver oil.

Vit-A (IU)	Fat (%)	Vit-D (IU)	Omega-3-fatty acids (%)
100,000.00	100.00	10,000.0	18.801

Experimental design: Rats were divided randomly into three groups, each group containing 10 rats (n =10). The first group was fed on basal diet and tap water. The second group was fed on the basal diet + 1% aqueous solution of lead acetate as drinking water. The third group was fed on basal diet + synbiotic fermented milk containing honey, garlic, ginseng, cod liver oil and chicory inulin against + 1% lead acetate as drinking water. The feeding experiment continued for five weeks. At the end of the experimental period, animals were anesthetized by exposure to an atmosphere of 100% diethyl ether and killed by decapitation. Blood samples were taken into plain tubes. Serum was harvested after centrifugation (3000 rpm) for 15 min. frozen at -20ºC for subsequent analyses.

Determination of glutathione-S-transferase (GST) in serum: The activity of GST was determined according to the method of Habig [12]. The method is based on the fact that the GST enzyme catalyzes the conjugation of glutathione with 1-chlor-2,4-dinitrobenzene (CDNB) and forms a complex which has an absorbance at UV (340 nm). The mean decrease of absorbance per min was calculated. To 100 µl serum, 100 µl glutathione solution, 10 µl fresh CDNB and 1290 µl phosphate buffer were added and gently mixed. The contents were incubated at room temperature for 1 h. The UV absorbance was measured using UV spectrophotometer at 340 nm. The UV absorbance of blank was obtained using assay mixture without serum as a control test. Enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 mol of product per min under condition of assay: Activity = A 340 nm/(9.6) x 1000 = M/min.

Other methods: Alanine and aspartate aminotransferase (ALT&AST) activities were determined according to the method described by Reitman and Frankel [13]. Chemical composition and sensory evaluation for the appearance, color, flavor and overall properties of synbiotic fermented milk were adopted from N.A.S.A [14] and analysed according to official methods [15]. Sensory evaluation was carried out using panelists with three categories: (++) good, (+) accepted, (-) unaccepted. Mean and standard error of the obtained data from each different experimental group were calculated and conducted according to the method described by Miller and Miller [16].

Results and Discussion

Sensory evaluation of the synbiotic-fermented milk is presented in Table 5. The sensory evaluation properties were found to have good scores and were acceptable for consumers.

The activities of serum GST are shown in Table 6. The obtained results showed that GST activity in sera of rat fed on synbiotic fermented milk were increased with mean values of 23.438 M/min

Table 5. Sensory evaluation of synbiotic fermented milk (++ good, + accepted).

Appearance	++
Color	+
Flavor	+
Overall	++

compared with negative and positive control with mean values of 12.01 and 13.95 M/min respectively.

Lead administration in drinking water for five weeks resulted in a slight increase in the activities of serum GST in the positive control rats and there was a significant increase in the activity of GST in the serum of rats receiving the synbiotic milk together with lead. It seems that administration of lead resulted in induction of the enzyme. It is well known that GST catalyses the formation of glutathione S-conjugates between glutathione and certain electrophilic substrates involved in GSH turnover in animals[17]. It has been found that GST mediates conjugation of various carcinogens and other electrophilic drugs in the liver and this is important in the protection of the body against these agents [18]. This protection was manifested in the decreased activities of the liver enzymes AST and ALT in rats receiving the synbiotic milk as presented in Tables 7 and 8 respectively. Administration of lead increased significantly ($p \leq 0.05$) the activities of these enzymes in the positive control, nevertheless, in rats receiving the synbiotic milk these enzymes returned almost to their normal values. This may indicate a possible role of the synbiotic fermented milk in protection against free radicals generated by lead. It has been evidenced that excess generation of reactive oxygen species in liver leads to hepatic injury as manifested by an increase in the activities of AST and ALT accompanied by tendency of apoptosis [19, 20]. The decreased activities of GST and the increase in the activities of AST and ALT may be contributing factors in lead poisoning. Kumar *et al.* found that paracetamol treatment to rats induced liver damage and significantly reduced GST and increased the activities of AST and ALT [21].

The beneficial effects of synbiotic milk can be attributed to many factors. Glutathione is the major intracellular redox buffering system involved in recycling of other antioxidants [22]. It also plays a role in a variety of reactions such as synthesis of proteins and DNA precursors. Glutathione depletion in liver and kidney is seen during severe oxidative stress in goats induced by lead administration. The depletion of GSH content also may lower GST, because GSH is required as a substrate for GST activity [23].

Glutathione is a tripeptide synthesized in the body from free amino acids, cysteine, glutamic acid and glycine. In many cancer epidemiological studies, it has been found that the major milk proteins, caseins and whey, have protective effect against cancer [24]. In this connection we can assume that milk proteins plus microbial proteins that are synthesized by probiotics can enrich the amino acids pool in the body and consequently increase glutathione and other protein precursors and synthesis. Similar assumption was proposed by McIntosh [25]. Busserolles *et al.* [26] noted that feeding a diet containing carbohydrates such as oligofructose and inulin such as chicory inulin caused enlargement of the caecum leading to a significant increase in the short-chain fatty acids (SCFA) pool. St-Onge *et al.* [27] and Busserolles *et al.* [26] showed that inulin, oligofructosaccaride and fermented dairy products significantly increased production of short-chain fatty acids and increased glutathione level in blood. Another advantage to the synbiotic fermented milk fed to rats in this study was that it contained honey which is a ready source of carbohydrates for microbial fermentation. Garlic, besides its well known health benefits, is a rich source of proteins (16.8%). The cod liver oil supplemented in this diet is rich in vitamin A and omega-3-fatty acids.

It can be concluded from this study that feeding synbiotic milk has many beneficial effects. It protects liver from damage caused by free radicals generated by lead contamination through many mechanisms. The speculated mechanism presented in this experiment is believed to be through more production and involvement of glutathione. Further investigation should be conducted to evaluate the effect of glutathione and enzymes involved in its formation.

Table 6. Glutathione-S-transferase in serum.

GST	Min	Max	Average	SD	CV%	Activity A / 9.6*1000 M/min
Negative control	0.076	0.17	0.115	0.0494	42.35	12.014
Positive control	0.096	0.18	0.134	0.0426	31.77	13.958
Synbiotic fermented milk	0.133	0.34	0.225	0.1052	46.74	23.438

Min Minimum value, Max Maximum value, SD Standard deviation, C.V. Coefficient of variation

Table 7. AST in serum (IU/L).

AST	Min	Max	Mean	SD
Negative control	150	180	160.0	17.32
Positive control	203	254	220.66	28.88
Synbiotic fermented milk	132	169	156.66	21.36

Min Minimum value, Max Maximum value, SD Standard deviation

Table 8. ALT in serum (IU/L).

ALT	Min	Max	Mean	SD
Negative control	19	26	23.00	3.60
Positive control	35	40	37.33	2.51
Synbiotic fermented milk	22	29	25.00	3.60

Min Minimum value, Max Maximum value, SD Standard deviation

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