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Rats with Lead Toxicity: The Impact of Fermented Milk Enhanced with some Prebiotic Sources on Biological and Biochemical Indicators

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ABSTRACT:

Changes in the gut's microbiota can influence pathways linked to metabolic risk factors for illness. So, the current study aims to investigate the effects of fermented milk with prebiotic supplementation on the treatment of changes to biological and biochemical parameters in rats treated with lead acetate. Forty-eight male albino rats weighing 110±10g were split into two main groups. The first group was used as a negative control, and the second group was given 1 g/L lead acetate in their drinking water for thirty days to make them toxic to lead. The second group was then split into seven subgroups. One was kept as the positive control group. The other six (G3, G4, G5, G6, G7, G8) were given fermented milk with different kinds of prebiotics (5% honey, 1% garlic, 1% ginseng, 1% cod liver oil, 1% chicory, and a 1% prebiotic mixture). We determined the biochemical and biological parameters of the tested groups. Using lead to treat the animals decreased their body weight, feed intake, feed efficiency ratio, hemoglobin, serum iron, and HDL-c. In contrast, their relative organ weight, liver enzymes, kidney functions, and lipid profile increased. Histopathological examination of liver and kidney tissues supported the biochemical results. Supplementation with prebiotics containing fermented milk helped alleviate toxic rats' biological and biochemical abnormalities. In conclusion, fermented milk containing prebiotic sources or their mixture may reduce the risk factor of lead toxicity.

Keywords: Rats, Lead, Prebiotics, Fermented Milk.

1. INTRODUCTION

Lead (Pb) is a non-essential heavy metal that is extremely toxic and has a negative impact on the majority of human and animal organ systems, leading to multisystem illness. It is regarded as a possible global environmental threat. Within the food chain, it experiences biomagnification. The FDA and the CDC suggest an Interim Reference Level of 12.5 µg/day for adults, while the Advisory Committee on Childhood lead Poisoning

Prevention (ACCLPP) sets an upper reference range value for children at 5 µg per deciliter (µg/dL) (1 and 2). pb poisoning can cause oxidative stress, which can change the expression of genes linked to reactive oxygen species (ROS). This can cause major, irreversible harm to the immune system, kidney, liver, hematological, reproductive, and gastrointestinal (GI) systems. The GI and respiratory systems are especially susceptible to Pb exposure; in children, the intestine absorbs 40–50% of ingested lead, while in adults, it absorbs only 10-15% (3). Pb absorption can be greatly impacted by the makeup of the food and certain nutritional deficits. The most popular treatment method for treating heavy metal toxicity in humans is chelation therapy, which involves administering chelating chemicals like dimercaptosuccinic acid (DMSA) and ethyl enediamine tetra acetic acid (EDTA) to promote the metal's elimination. These chelators do, however, come with several drawbacks. Thus, creating novel dietary intervention techniques is crucial to preventing harm from lead exposure (1 and 4).

Probiotic sources are utilized in therapy techniques. Probiotics are live microorganisms that have been demonstrated to provide numerous health advantages for people, including as reducing inflammation, oxidative stress, and GI disorders and supporting the maintenance of a balanced microbial community in the gut (5). Fermented foods contain Lactobacillus and Bifidobacteria, which are beneficial bacteria found in the gastrointestinal tract (GIT). The ability of these probiotic bacteria to excrete heavy metals and bind to other toxic compounds like cyanotoxins and dietary mutagens has been shown to have remarkable detoxifying effects on the human gut from heavy metals like cadmium and lead. They also boast the highest antioxidant properties among gut microbes (6). Many studies, both in vivo and in vitro, have examined how to change the gut microbiota's composition by boosting the growth of probiotic lactobacilli and bifidobacteria. These studies have mostly concentrated on prebiotic fructo-oligosaccharides (FOS), which are found in plant-based foods like chicory, garlic, onion, jerusalem artichokes, bananas, wheat, asparagus, and leek (7). Prebiotics, like inulin, can help probiotics thrive in the colon. They are frequently taken as dietary supplements or as part of fermented foods like yogurt, soy yogurt, and soymilk (8).

Honey has been used medicinally for a very long time, and one of its many uses is as a digestive tonic as antibacterial, anti-inflammatory, wound healing, and antioxidant qualities. Because honey includes non-digestible oligosaccharides, there is increasing evidence from animal, in vitro, and pilot human research that some honey varieties may have prebiotic properties that can improve gut flora (9).

Garlic (Allium sativum) contains active compounds with antibacterial properties, including allicin and other sulfur-containing components. At a 1.0% level, garlic can be added to feed as a natural prebiotic to enhance growth and have an antibacterial effect on a variety of Gram-positive and/or Gram-negative bacteria, including Proteus species, Staphylococcus, and Mycobacteria. Research has shown that garlic (10).

Chicory, or Cichorium intybus, L., is a significant perennial herb plant in the Asteraceae family that is used medicinally. It has high concentrations of flavonoids, fructooligosaccharides, coumarins, inulin, and several vitamins. As an anti-hepatotoxic, anti-ulcerogenic, anti-inflammatory, diuretic, depurative, alexiteric and tonic substance, chicory was utilized. Specifically, one of the advantageous elements of chicory that helps control appetite and the conversion of lipids to glucose is inulin. It has also been demonstrated that chicory can limit the growth of gut pathogenic bacteria and encourage the growth of beneficial germs (11).

Ginseng (Panax quinquefolius, L.), the root of plants of the Panax genus, as a herbal remedy. It is generally characterized by the presence of ginsenosides, acidic polysaccharides, mineral elements, and dietary fiber. These substances support gut health in humans by thickening the contents of the intestinal tract, promoting the growth of gut microbes, and increasing stool weight and regularity. They also have antioxidant, anti-proliferative, and neuroprotective properties, as well as improved blood circulation and immune system performance (12).

Cod liver oil has a high concentration of essential fatty acids, particularly n-3 HUFA, it serves as the primary lipidic supply for the diets of marine carnivorous fish. As a result, omega 3 fatty acids enhance the general population of beneficial bacteria in the digestive system and boost the advantages of consuming probiotics (13). Treating ulcers, especially those in the stomach and intestines, may be aided by cod liver oil. The current investigation was to find out how some prebiotic sources combined with fermented milk could reduce the biological and biochemical indicators of Pb toxicity in rats (14).

The purpose of this study was to evaluate how prebiotic-fortified fermented milk influenced the biological and biochemical parameters of lead-intoxicated rats.

2. Material and Methods

2.1. Materials

Chemicals, kits, and lead acetate were acquired from El-Gomhorya Company in Cairo, Egypt. A basal diet was received from Technogene Chemical Co., Dokki, Egypt, and included casein as the primary source of protein, cellulose, a mixture of vitamins, salt, methionine, and corn starch.

The ginseng, garlic, chicory, honey, and other ingredients were sourced from

Menoufia University's Faculty of Agriculture in Shebin El-Kom. Cod liver oil (Gadus morhua) was bought from Shebin El-Kom City, Menoufia Governorate, Egypt, at the local market.

Probiotic yoghurt starter culture contains Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium bifidum obtained from Agriculture Faculty Ain- Shams University.

Forty-eight adult male Sprague-Dawely albino rats, weighing 110±10g and three months old, were acquired from the Medical Insects Research Institute in Dokki, Cairo, Egypt.

2.2. Methods

2.2.1. Lead toxicity induction:

For a duration of thirty days, the rats were gavage with 1 g/L lead acetate once a day in drinking water (15).

2.2.2. Preparation of garlic, chicory, and ginseng

After being cleaned, the plants were dried for eight hours at 60 °C in an oven (16). Each dried plant (1 kilogram) was ground into a powder and extracted using 10 L of water after being heated to reflux for an hour. Next, under low pressure, the decoction was filtered and concentrated (17).

2.2.3. Preparation of fermented milk

The procedures for fermented milk were followed by pertormed as the method of (18), After 30 minutes of heating at 85°C, milk was cooled to 40°C, inoculated with 1:1:1 strains of Lactobacillus acidophilus, Bifidobacterium, and Streptococcus thermophilus, and incubated at 42°C for 4–8 hours. Following coagulation, the curd's pH was measured, it was agitated in an electric blender, and it was refrigerated at 4-6±2°C to produce a solid content of roughly 12%. Fermented milk was combined with various probiotic sources at 1%, except for honey, which was combined at 5%, to create synbiotic fermented milk.

2.2.4. Experimental design

The Science Research Ethics Committee of the Faculty of Home Economics accepted the research protocol MUFHE/S/NFS/14/24).

Forty-eight adult male Sprague Dawley strain albino rats, weighing 110±10, were procured from the Medical Insects Research Institute, located in Dokki, Cairo, Egypt. Fine components were used per 100g to produce the basic diet. The nutritional makeup of the food was as follows: 67% maize starch, 13% casein, 10% corn oil, 5% fiber (19), 4% salt formula (20), and 1% vitamin formula (21). The Menoufia University Faculty of Home Economics, Shebin El-kom, Menoufia Governorate, Egypt, was the site of the experiment.

Forty-eight albino rats, all of which were roughly three months old, and they were all weighted according to the previously mentioned method. Rat cages were maintained in a room with normal health conditions and a temperature of 25°C. After a week of a basal diet to allow for acclimatization, all the rats were split into eight groups of six rats each, as follows:

- Group 1: As a negative control group, rats were provided a basal diet.

-Group 2: As a positive control group, rats were given 1 g/L lead acetate in their drinking water every day for thirty days.

-Group 3: Rats were fed 10% fermented milk mixed with 5% meal honey and given 1 g/L lead acetate daily in their drinking water.

- Group 4: Rats had 10% fermented milk supplemented with 1% diet garlic and were given 1 g/L lead acetate daily in their drinking water.

- Group 5: Rats received 10% fermented milk supplemented with 1% ginseng from the diet and were administered 1 g/L lead acetate daily in their drinking water.

- Group (6): Rats were fed 10% fermented milk supplemented with 1% cod liver oil from the meal and given 1 g/L lead acetate daily in their drinking water.

-Group 7: Rats received 10% fermented milk supplemented with 1% chicory from the meal, and they were given 1 g/L lead acetate daily in their drinking water.

-Group (8): Rats were fed 10% fermented milk supplemented with 1% probiotic sources from the diet and given 1 g/L lead acetate daily in their drinking water.

To prevent food loss and contamination, the meals were given to the rats in specific non-scattering feeding cups. Each of the groups was housed in a single wire cage. Rats were given access to tap water through glass tubes that protruded through wire cages from bottles that were flipped and held to one side of the cage. Both at the start and finish of the trial, the rats were weighed. Each rat's organs, liver, spleen, lungs, heart, and kidney were quickly removed and weighed individually.

2.2.5. Biological evaluation

Every week, the weight of each rat was measured, and each day's feed intake was computed. Following the conclusion of the study, body weight gain (BWG), feed efficiency ratio (FER), and relative organ weight (ROW) were used to evaluate the various diets biologically according to the subsequent equations of (22).

$$\text{BWG} = \text{Final weight} - \text{Initial weight}$$

$$\text{FER} = \frac{\text{Feed Intake (g)}}{\text{Gain in body weight (g)}}$$

$$\text{Relative organ weight (ROW)} = \frac{\text{Organ weight (g)}}{\text{Final body weight(g)}} \times 100$$

2.2.6. Blood sampling

At the conclusion of the trial, blood samples were taken from each of the groups following a 12-hour fast. Blood was drawn into a dry, clean centrifuge tube using the retro-orbital method using micro capillary glass heparinized tubes. The tube was then allowed to coagulate in a water bath (371 C) at room temperature for thirty minutes. In accordance with (23) procedure, the blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum. The serum was carefully drawn out and placed into sterile, tight-fitting plastic tubes. It was then refrigerated at -20°C until it was time for analysis.

2.2.7. Assay of serum biochemical parameters

Using 2, 4-dinitrophenylhydrazine as a substrate, the Randox reagent kit was used to assess the activities of serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in accordance with the methodology outlined by (24). The following procedures of (25, 26, and 27) were used to determine total protein (TP), albumin (Alb), and globulin (Glb),

Urea, uric acid, and creatinine were measured in accordance with (28, 29, and 30) as kidney function markers.

Heparinized blood was analyzed for estimation of hemoglobin (HB) which was assessed by (31) and serum iron was carried out according to the methods of (32).

Serum total cholesterol, triglycerides, and HDL-c were measured using the procedure outlined by (33, 34, and 35). According to (36), LDL-c and VLDL-c were computed in mg/dl using the following formula:

Total cholesterol – (HDL-c + VLDL-c)

VLDL-c (mg/dl) = Triglycerides / 5 LDL-c (mg/dl).

2.2.8. Histological study

All experimental groups provided small liver and kidney tissues, which were then fixed in 15% neutral buffered formalin, dehydrated in increasing ethanol concentrations (70, 80, and 90%), cleaned in xylene, and embedded in paraffin (37).

2.2.9. Statistical analysis

With the assistance of the statistical software program SPSS for Windows (Version 18), data were statically analyzed. According to (38) the one-way analysis of variance (ANOVA) test was used to assess the significance of differences between more than two groups.

3. Results and Discussion

Table 1 indicate that the treatment of fermented milk fortified with various probiotic sources decreased the means of body weight increase, feed intake, and feed efficiency in rats with Pb toxicity when compared to the negative control group. When compared to the positive control group and group (3), which had a 5% honey treatment, had the greatest values, while group (7), which received 1% chicory as food, had the lowest values. Regarding body weight and FER, there were no significant differences among groups 6 and 8. These results disagreed with (39) due to the dose, experimental animals, and duration of the study; however, group (3) (Pb toxicity and treated with honey) revealed an increase in body weight compared with positive one, which was consistent with (9). Nabil et al., (40) reported that loss of body weight was caused by impaired intestinal absorption of some essential trace elements. The outcome might be associated with the fact that honey is necessary to preserve the body's hemostasis and regular metabolic functions. When compared to the positive group, Group 4 (lead acetate plus garlic) showed an increase in body weight. The rats which were exposed to lead toxicity had a reduction in feed intake, body weight gain and feed efficiency (41). This conclusion was in line with that of (10), who demonstrated that garlic is a great source of growth-related minerals such as magnesium, phosphorus, manganese, copper, iron, and zinc. Garlic may also be utilized as a natural prebiotic in feed at a level of 1.0% to enhance growth and control hunger. Chicory, cod liver oil, ginseng, and other sources of prebiotics were found to be beneficial in that they contained significant amounts of fructooligosaccharides, inulin, coumarins, flavonoids, and several vitamins. These substances can both stimulate the growth of beneficial microbes and inhibit the growth of gut pathogenic bacteria (12).

Table (1): The effect of fortified fermented milk with the used experimented of prebiotics on BWG, feed intake (FI) and (FER) for lead toxicity rats

	B.W.G (g/28day)	FI (g/day)	FER (rat/day)
(Negative control) (G1)	38.98 ±1.01 a	14.50±0.09 a	0.096 ±0.002 a
Positive control (G2)	8.97 ±0.04 f	4.93 ±0.11 g	0.065 ±0.001 g
Fermented milk with 5% honey (G3)	23.82 ±0.87 b	9.56±0.05 b	0.089 ±0.002 b
Fermented milk with 1% Garlic (G4)	18.25 ±0.29 c	7.67 ±0.16 c	0.085±0.003 c
Fermented milk with 1% ginseng (G5)	11.49 ±1.04 e	5.33 ±0.24 f	0.077 ±0.002 e
Fermented milk with 1% cod liver oil (G6)	13.12 ±0.56 d	5.93 ±0.04 e	0.079 ±0.001 d
Fermented milk with1%chicory (G7)	9.99 ±0.88 f	5.03 ±0.28 f	0.071 ±0.001 f
Fermented milk with 1% materials mixture (G8)	14.74 ±1.65 d	6.50 ±0.04 d	0.081 ±0.002 d
L.S.D	1.86	0.34	0.003

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05).
BWG: body weight gain, FI: feed intake, FER: feed efficiency ratio

The current findings in Table (2) demonstrated that administering lead acetate had an impact on the relative weight of the five organs under examination (liver, heart, kidneys, lungs, and spleen). When compared to the negative control group, there was a significant reduction in these parameters, but there was a significant rise in the weight of the organs following the experimental period, either in terms of organ weight or the ratio% relative to final body weight. Between the groups of chicory and ginseng, as well as between the groups of garlic and honey, there are no significant differences. Lead acetate has the chemical formula of an organic salt. Pb that is dissolved in water will undergo hydrolysis. It is the hydrolysis process that produces both positive and negative ions. Pb will ionize in the solution to form the anion C2H3O2- and the cation Pb2 +. The outer layers of Pb2 + cations include unbound atoms. Pb2 + transforms into free radicals as a result of the free atom attempting to bond to other organ molecules in order to complete the outer layer and become more stable. By attaching to the PUFA (polyunsaturated fatty acid) liver cell membrane and starting cell membrane lipid peroxidation, Pb2 + in the liver will attempt to finish the outer layer. Membrane fluidity, membrane cross-linking, and membrane structure and function will all be impacted by this peroxidation. Following their reaction with the membrane cell, the sodium and potassium channels were broken, causing extracellular water to enter the cell and leading to hydrophic degeneration. Pb also caused coagulative necrosis cells in the kidney and spleen, whose nuclei seemed to be fragmented, followed by the appearance of inflammatory cells in the necrotic area (42). Consequently, administering pebiotics such as honey, garlic, and others can lessen the degree of cell damage brought on by exposure to lead acetate. Prebiotics can be used as a preventative measure to lessen and neutralize free radicals in the body. The sources of tested prebiotics are antioxidant-rich sources that contain polyphenol compounds like flavonoids. These compounds work by donating one electron to unpaired electrons in free radicals, thereby inhibiting oxidation reactions and reducing their number. By attaching to free radicals and reducing their damaging effects on the liver and other essential organs, flavonoids may also have an influence on preventing damage to these organs (12) After feeding on chicory leaves powder, the organ weight dramatically dropped (p≤0.05) (43).

Table (2): The effect of fortified fermented milk with the used experimented of prebiotics on relative organs weight (liver, heart, kidneys, lungs and spleen) for lead toxicity rats

Groups	Relative liver Weight (%)	Relative heart weight (%)	Relative kidneys weight (%)	Relative lungs weight (%)	Relative spleen weight (%)
Negative control (G1)	3.93 f±0.01	0.30f ±0.01	1.01f±0.01	0.84 f±0.10	0.47f ± 0.008
Positive control (G2)	4.96a±0.02	0.51a±0.02	1.87a±0.02	1.01a ± 0.001	0.67a ± 0.01
Fermented milk with 5% honey (G3)	4.11e ±0.04	0.34e±0.01	1.43e±0.02	0.85e ± 0.009	0.52e ± 0.006
Fermented milk with 1% Garlic (G4)	4.23e±0.14	0.35e±0.02	1.46e±0.01	0.86e±0.007	0.53 e± 0.01
Fermented milk with 1% ginseng (G5)	4.76b±0.01	0.46b±0.01	1.78b±0.04	0.97b±0.005	0.62b ±0.008
Fermented milk with 1% cod 4 liver oil (G6)	4.60 c ±0.13	0.43c±0.03	1.72c±0.02	0.94c±0.02	0.58 c ±0.005
Fermented milk with1%chicory (G7)	4.80 b ±0.11	0.47b±0.01	1.81b±0.01	0.98b±0.01	0.63 b ±0.007
Fermented milk with 1% materials mixture (G8)	4.41 d ±0.09	0.39d±0.02	1.65d±0.03	0.91 d ±0.01	0.55d ±0.01
L.S.D	0.15	0.03	0.04	0.02	0.01

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05).

When assessing liver function in inebriated animals compared to negative control rats, measures such as plasma AST, ALT activity, and ALP levels are used (Table 3). These findings demonstrated that pb strongly and significantly raised AST, ALT, and ALP activity. After the trial, data from rats treated with various prebiotic sources and pb-intoxicated rats showed a significant decrease in their values compared to the positive control group. In comparison to the positive control group, the tested mixture and honey and garlic exhibited significant reduction percentages (42.24, 58.47, and 28.76%, respectively). The effects of honey, garlic, and the material mixture were greater than those of the other examined probiotic sources, while they were still significantly reduced. The damage to the liver cells of Pb2+-intoxicated mice was revealed by the current results of the liver function measures (ALT, AST, and ALP). These findings concur with earlier research that found pb to have hepatotoxic effects. Together with elevated liver microsomal membrane fluidity, free radical production, and changes in the liver tissue histogram, there is also an increase in plasma ALT and AST activity (40).These findings are consistent with earlier research by (44), which found that the organic therapeutic biomolecular agents found in honey, including kaempherol, quercetin, chrysin, luteolin, apigenin, and vanillic acid, significantly reduced ALT and AST levels. Furthermore, several studies have documented the significance of these chemical compounds in hepatic and biliary therapy. By preserving the integrity of the liver's biomembrane, lowering the levels of free radical species, and controlling the liver's metabolic processes, the organic compounds most likely lower these liver enzymes (45). Garlic's sulfur compounds, which include S-allylcysteine, vinyldithiins, ajoene, allylpropyl disulfide, diallyl trisulfide, and S-allylmercaptocysteine, are its potentially active chemical ingredients Through the suppression of oxidative stress, the reduction of lipid peroxidation indicators (malondialdehyde), and the modification of the hepatic antioxidant system. A significant drop in the level of liver enzymes following garlic supplementation. The other prebiotics under evaluation reduced liver enzyme levels and contained polysaccharides with scavenging, anticancer, and anti-complementary properties. Pectic polysaccharides, which are sugars soluble in water, consist of the covalently bonded domains of rhamnogalacturonan (RG)-I and II and homogalacturonan (HG). Administration with chicory leaves powder (ChLP) at concentration of 2.5, 5 and 10% significantly ($p \le 0.05$) reduced the level of serum AST, ALT, and ALP (46). Administration with (ChLP) at concentration of 2.5, 5 and 10% significantly ($p \le 0.05$) reduced the level of serum AST, ALT and ALP (43).

Table (3): The effect of fortified fermented milk with the used experimented of prebiotics on AST, ALT, and ALP for lead toxicity rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative control (G1)	40.07±1.56 h	38.63±2.63 h	71.47±2.93 h
Positive control (G2)	98.08±2.93 a	92.34±3.14 a	142.11±2.03 a
Fermented milk with 5% honey (G3)	56.65±0.07 g	50.56±0.52 g	89.66 ±4.18 g
Fermented milk with 1% Garlic (G4)	61.89 ±0.71 f	59.45 ±1.43 f	95.67 ±3.51 f
Fermented milk with 1% ginseng (G5)	82.14 ±2.55 c	79.15 ±2.35 c	126.83 ±2.44c
Fermented milk with 1% cod 4 liver oil (G6)	76.57 ±3.04 d	73.81 ±1.04 d	117.56 ±1.29 d
Fermented milk with1%chicory (G7)	88.93 ±0.57 b	83.57 ±0.74 b	133.37 ±5.02 b
Fermented milk with 1% materials mixture (G8)	69.87 ±1.68e	65.667±4.01 e	108.4667 ±4.28 e
L.S.D	3.94	4.05	5.39

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05).). AST: aspartate aminotransferase, ALT: alanine transaminase, ALP: alkaline phosphatase.

Table 4 revealed that while the levels of TP, Albumin and Glb in the negative control group were within the normal range, the exposure group's (positive control group) level of this parameter was considerably lower than that of the non-exposure group (P ≤ 0.05). The evaluated parameter levels underwent significant changes in the groups exposed to and treated with prebiotic sources, with the prebiotic source determining the degree of improvement. The honey group exhibited considerably higher values for Glob, Alb, and TP than the other treatment groups (P ≤ 0.05), whereas the differences between the garlic and material mixture groups were not statistically significant. The garlic group was followed by the pb-containing combination material groups. Their levels, however, had the least impact with nonsignificant changes in the ginseng and chicory groups. The serum TP serves as an indirect gauge of protein status, reflecting significant alterations in liver function (47). The co-administration of honey and vitamin demonstrated the greatest increase in total protein. This indicates that the active ingredients in honey can enhance the impact of other substances on the total amount of proteins. Vitamin E is present in honey. It suggests that the amount of the antioxidant vitamin that protects all protein may have been enhanced (45). Garlic's greatest impact was attributed to its high concentration of polysaccharides, proteins, lipids, vitamins, flavonoids, trace elements (including selenium), and organosulfur compounds. The primary active ingredients in garlic are called polysaccharides (GPs), and they have a range of biological properties including immunomodulatory, anticancer, hepatoprotective, and anti-inflammatory properties. It shields the liver cells from free radical damage, which promotes the entry of these parameters into the bloodstream (49).

Table (4): The effect of fortified fermented milk with the used experimented of prebiotics on TP, Alb and Glob level for lead toxicity rats

Groups	T. Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)
Negative control (G1)	11.64a±0.153	4.57a ± 0.21	7.07 a ± 0.28
Positive control (G2)	4.82f±1.258	1.65f ± 0.01	3.17f ± 0.17
Fermented milk with 5% honey (G3)	8.88 b ±0.436	3.42b ± 0.11	5.46b ± 0.08
Fermented milk with 1% Garlic (G4)	8.30 c ±0.929	3.18c ± 0.07	5.12c ± 0.21
Fermented milk with 1% ginseng (G5)	6.19 e ± 2.166	2.03e ± 0.08	4.16e ± 0.07
Fermented milk with 1% cod liver oil (G6)	7.12d ±0.737	2.59d ±0.19	4.53d ± 0.12
Fermented milk with1%chicory (G7)	5.82e ±2.845	1.97 e ±0.19	3.85e ± 0.17
Fermented milk with 1% materials mixture (G8)	7.81c ± 0.945	2.89c ± 0.16	4.92c ± 0.05
L.S.D	0.53	0.23	0.31

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05). T. protein= Total protein.

Table 5 data showed that the pb-treated rats had significantly higher levels of urea, creatinine, and uric acid (P < 0.05) than the negative control group (56.58%, 50.48%, and 84.85%, respectively). However, the evaluated parameters were reduced in the treated rats given varying prebiotic sources, with the magnitude of the decrease varying according to the prebiotic source type. The groups that showed no significant difference between each other were the chicory and ginseng groups, while the honey group had the largest decline. The effects of the other probiotic sources were notably less than those of the material mixture, garlic, and honey. Adverse effects on the kidneys might occur from prolonged exposure to pb. Acute Pb nephropathy is defined by the physical appearance of degenerative alterations in the tubular epithelium and nuclear inclusion bodies containing Pb protein complexes, as well as a broad impairment of tubular transport mechanisms (Fanconi syndrome). Because honey contains minor compounds that may contribute to its antioxidant activity, such as sugars, proteins, organic acids, amino acids, flavonoids, phenolic acids, and Maillard reaction products, it significantly reduced the elevation of kidney functions caused by toxic heavy metals while also elevating the antioxidant status (45). Garlic's allicin prevented oxidative damage to kidney tissue and the nephrotoxic effects of CsA. It also greatly lowered the reactions of isolated bladder rings to ACh (49).

Table 6 shows that there was substantial contribution (P ≤ 0.05) drop in the serum iron and hemoglobin (HB) levels in the pb-toxicities rats as compared to the negative control group. With nonsignificant difference, the effects of the chicory and ginseng treatments were smaller than those of the other treatments, which differed significantly.

Table (5): The effect of fortified fermented milk with the used experimented of prebiotics on urea, creatinine, and uric acid for lead toxicity rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
Negative control (G1)	25.33g ± 1.53	0.450g ± 0.04	4.67g ± 0.05
Positive control (G2)	58.34a ± 2.65	2.97a ± 0.03	9.43a ± 0.31
Fermented milk with 5% honey (G3)1	33.27f ± 2.46	1.07 f ± 0.07	5.24 f ± 0.17
Fermented milk with 1% Garlic (G4)2	36.41e ± 2.96	1.25e ± 0.09	5.97e ± 0.23
Fermented milk with 1% ginseng (G5)5	47.31 b ±1.89	2.07 b ±0.04	7.09 b ±0.15
Fermented milk with 1% cod 4 liver oil (G6)	42.67c±2.50	1.98c±0.01	6.77 c ±0.21
Fermented milk with1%chicory (G7)6	48.03 b ±2.08	2.11 b ±0.08	7.11 b ±0.30
Fermented milk with 1% materials mixture (G8)	39.77 d ±1.85	1.68 d±0.05	6.37 d ±0.28
L.S.D	1.25	0.11	0.32

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05).

Comparing the honey group to the positive control group, the material mixture treatment resulted in a significant rise in both HB and serum iron levels. The other probiotic therapy showed significant difference in its effects from the positive control and was less effective than both honey and garlic.

Blood pb and HB had a strong negative correlation, according to the earlier study. Furthermore, it has been hypothesized that the underlying cause of pb-induced anemia (hypochromic anemia) is a vicious cycle of rising Pb toxicity and deteriorating iron deficiency resulting from competition between iron and pb for absorption in the small intestine. Additionally, they discovered that Pb decreased red blood cell survival and interfered with heme production (42). Serum iron and HB levels improved because of the honey treatment. High concentrations of iron, manganese, and copper all necessary for the synthesis of HB can be found in honey. Ancient literature states that honey helps to maintain the ideal level of red blood cells and HB, making it a traditional treatment and preventative for anemia (45).

The findings demonstrated that while the HDL levels in the pb-treated group were lower than those in the non-treated group (P ≤0.05), the lipid profile levels in the pb-treated group were greater than those in the non-treated group (P ≤0.05). Additionally, the pb groups' levels of LDL-c, VLDL-c, TC, and TG dropped when they were supplemented with fermented milk containing prebiotic sources, but their HDL levels rose with time (Table 7). The garlic and honey groups had the strongest effects, while the chicory group had the least, and neither group's difference from the positive control group was statistically significant. Previous findings have shown that aberrant lipid profiles are linked to obesity or overweight and are a significant cause of cardiovascular disease. HDL supplies the necessary protein components to trigger lipoprotein lipase, releasing fatty acids that can be converted into energy via the **β**-oxidation pathway.

Table (6): The effect of fortified fermented milk with the used experimented of prebiotics on serum iron and HB levels for lead toxicity rats

Groups	Hemoglobin (mg/dl)	Serum iron (mcg/dL)
Negative control (G1)	12.46a ± 0.04	60.45a ± 2.11
Positive control (G2)	8.27e ± 0.43	39.83e ± 1.167
Fermented milk with 5% honey (G3)	10.07b ± 0.08	48.51b ± 3.729
Fermented milk with 1% Garlic (G4)	9.76b ± 0.12	47.35b ± 5.151
Fermented milk with 1% ginseng (G5)	8.89d ± 0.08	43.12d ± 3.143
Fermented milk with 1% cod liver oil (G6)	9.01c ± 0.35	44.71 c ± 2.784
Fermented milk with1%chicory (G7)	8.67d ± 0.41	42.11d ± 3.717
Fermented milk with 1% materials mixture (G8)	9.41c ± 0.21	45.65c ±1.572
L.S.D	0.45	1.16

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05).

Most notably, because HDL is an antioxidant, it can prevent LDL from oxidizing and the atherogenic effects of oxidized LDL. Few research has looked into the relationships between serum lipid profiles and blood pb levels. pb levels have a positive relationship with lipid profile levels, except for HDL-c. These findings indicate that the structure and makeup of the gut microbiota can shift significantly when metabolic disorders worsen (42). Probiotic supplements greatly mitigated the effects of pb, which raised levels of triglycerides, LDL-c, and total cholesterol while lowering HDL-c. Certain probiotic strains have been shown to help lower TC, TG, and LDL-C levels while raising HDL-C levels in the host. By binding cholesterol, prebiotics can lessen the amount of cholesterol that the intestine absorbs. Prebiotics may lessen the enterohepatic circulation of bile salts, which would need the consumption of cholesterol for the liver's resynthesis of bile salts and a decrease in blood cholesterol levels to control the subjects' gut flora. The probiotic treatment's dosage, which has a significant impact on lipid abnormalities (50).

Furthermore, diabetic rats revealed that administration of fermented milk (Rayeb); kefir milk and their mixtures improve serum lipid profile such as HDL-c, LDL-c, and VLDL-c, levels in rats. The best results recorded were especially for 3ml mixtures fermented milk, besides the fact that it has so many health benefits (51).

Table (7): The effect of fortified fermented milk with the used experimented of prebiotics on lipid profile levels for lead toxicity rats

Group	Chol. (mg/dl)	T.G (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Negative control (G1)	85.11g ± 2.11	100.78h ± 4.04	40.89a± 0.09	24.06h ±0.65	20.16h ± 0.95
Positive control (G2)	140.57a ± 3.91	218.87a ± 2.97	25.87h ± 0.86	70.93a ±1.13	43.77a ± 0.54
Fermented milk with 5% honey (G3)	113.41f ± 1.56	170.32g ±3.11	38.02 b ± 0.48	41.33g ±1.02	34.06 g ± 0.68
Fermented milk with 1% Garlic (G4)	119.76e ± 2.61	179.56 f ± 3.02	36.93 c ± 0.44	46.92f± 1.01	35.91 f ± 0.71
Fermented milk with 1% ginseng (G5)	132.94b ±1.81	200.45c ±4.87	31.03f±0.29	61.82 c ±0.22	40.09 c ±0.88
Fermented milk with 1% cod liver oil (G6)	127.23 c ±2.45	193.82 d±5.02	33.86 e ±0.75	54.61 d ±0.64	38.76d±0.91
Fermented milk with 1% chicory (G7)	138.23a±2.91	210.82b ±3.92	28.73g ±0.47	67.34 b ±1.03	42.16 b ±0.86
Fermented milk with 1% materials mixture (G8)	122.14d±3.67	185.43 e ±5.21	35.04d ±0.17	50.01e± 0.78	37.09 e ±0.95
L.S.D	4.34	5.24	1.03	1.44	1.25

Values mean ± SD. Values in the same column sharing the same superscript letters are not statistically significantly different (p≤ 0.05). Chol.: cholesterol, T.G: Triglycerides, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, VLDL: very low-density lipoprotein cholesterol.

Histopathological of liver

Group (1), the negative control, had liver histology that showed normal hepatic lobule architecture, a normal central vein, and normal hepatocytes (Photo 1). In contrast, the liver of the rats in group (2) (the positive control) showed intravascular leucocyte penetration, Kupffer cell activation, and hepatocyte vacuolar degeneration (Photo 2). With only Kupffer cells activated, the liver from group (3) fermented milk with 5% honey showed no histological alterations (Photo 3). Examined sections from group 4 (fermented milk containing 1% garlic), however, did not exhibit any histopathological alterations despite a minor Kupffer cell activation (Photo 4). Group 5 (fermented milk with 1% ginseng) showed a little activation of Kupffer cells and a small congestion of hepatic sinusoids (Photo 5). Additionally, group 6's liver (fermented milk with 1% cod liver oil) showed a modest activation of Kupffer cells (Photo 6). In contrast, liver slices from rats in group (7) (fermented milk containing 1% chicory) showed mild activation of Kupffer cells and binucleation of hepatocytes (Photo 7). Furthermore, rats from group (8), whose livers were fermented milk containing a 1% mixture of honey and other materials, did not exhibit any histological alterations, except for a little degradation of some hepatocytes' vacuoles (Photo 8). According to studies by (6 and 12), flavonoids found in honey and garlic are regarded to have the ability to bind to free radicals and prevent damage to the kidneys and other important organs. By giving one electron to unpaired electrons in free radicals, flavonoids can suppress oxidation reactions by reducing their number. This is achieved through a process known as radical scavenging.

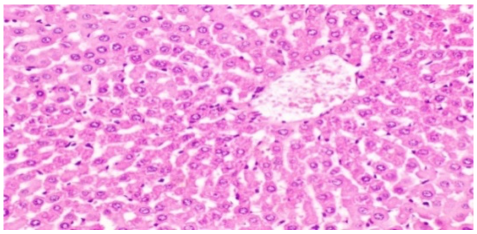


Photo (1): Photomicrograph of rat's liver from group 1 (negative control) (H & E X 400).

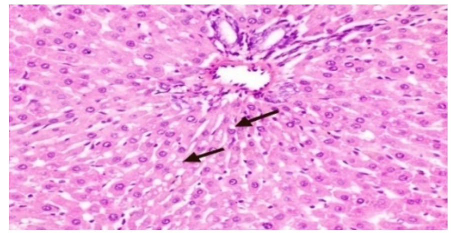


Photo (2): Photomicrograph of rat's liver from group 2 (positive control) (H & E X 400).

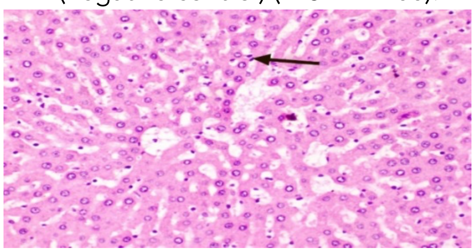


Photo (3): Photomicrograph of rat's liver from group 3 (fermented milk with 5% honey) (H & E X 400).

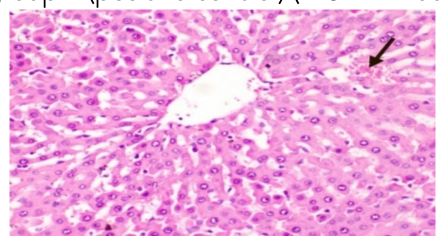


Photo (4): Photomicrograph of rat's liver from group 4 (fermented milk with 1% garlic) (H & E X 400).

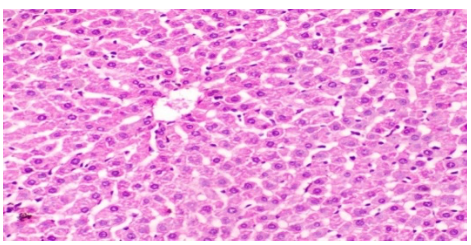


Photo (5): Photomicrograph of rat's liver from group 5 (fermented milk with 1% ginseng) (H & E X 400).

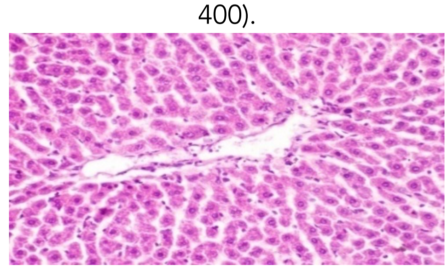


Photo (6): Photomicrograph of rat's liver from group 6 (fermented milk with 1% cod liver oil) (H & E X 400).

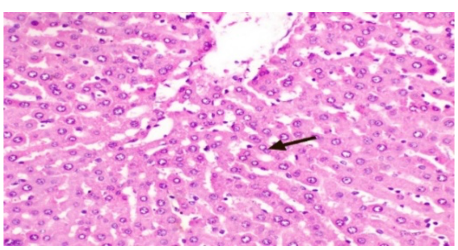


Photo (7): Photomicrograph of rat's liver from group 7 (fermented milk with 1% chicory) (H & E X 400).

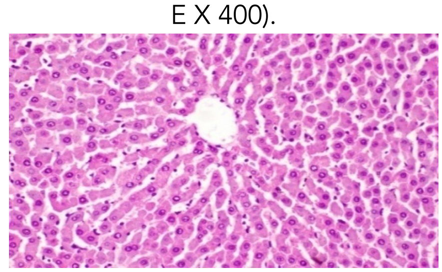


Photo (8): Photomicrograph of rat's liver from group 8 (fermented milk with 1% mixture of honey and other materials) (H & E X 400).

Histopathological of kidney

The kidneys in the negative control group (1) (negative control) showed normal renal parenchymal histology, which includes normal renal cortex and renal medulla (Photo 9). Conversely, kidney slices from rats in group (2) (positive control) demonstrated constriction of renal blood vessels and pronounced vacuolization of the epithelium lining renal tubules (Photo 10). Sections from group (3) (fermented milk with 5% honey) had a modest renal blood vessel congestion but no other histological changes (Photo 11). In addition, the kidneys of the rats in group 4 (fermented milk containing 1% garlic) showed no histological changes other than renal blood channel congestion (Photo 12). In contrast, slices from group (5) (fermented milk containing 1% ginseng) showed evidence of a mild vacuolar degeneration in the renal tubule epithelium (Photo 13). In addition, the kidneys of the rats in group (6), which were fed fermented milk containing 1% cod liver oil, showed minor vacuolar degradation of the epithelium lining some renal tubules and congestion of renal blood vessels (Photo 14). Rat kidneys from group (7) (fermented milk containing 1% chicory) revealed a mild vacuolar degradation of the renal tubule epithelium (Photo 15). Nevertheless, no histopathological changes with renal blood vessel congestion were seen in the sections from group (8) (fermented milk with a 1% mixture of honey and other components) that were investigated (Photo 16). pb-induced liver problems were demonstrated by (42) who also distinguished between hepatocellular necrosis, liver microsomal membrane fluidity, and changes in the liver tissue histogram (40). According to (44). Honey contains organic medicinal biomolecular agents that reduce free radical species and preserve the integrity of the biomembrane, including kaempherol, quercetin, chrysin, luteolin, and apigenin.

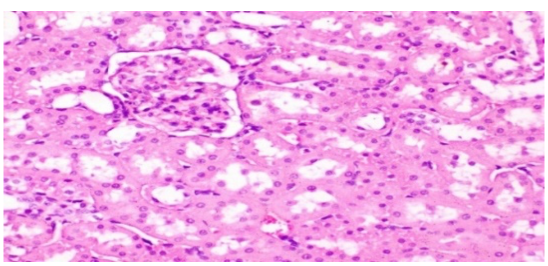


Photo (9): Photomicrograph of rat’s kidney from group 1(negative control) (H & E X 400).

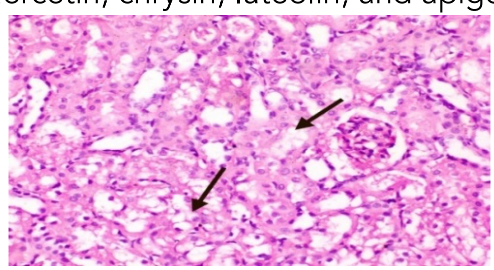
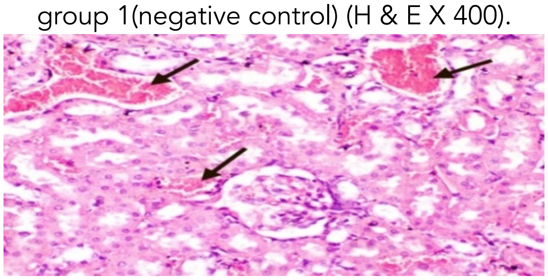


Photo (10): Photomicrograph of rat’s kidney from group 2 (positive control) (H & E X 400).



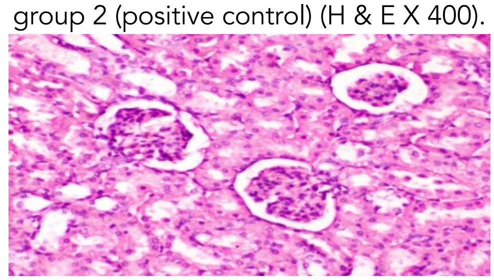
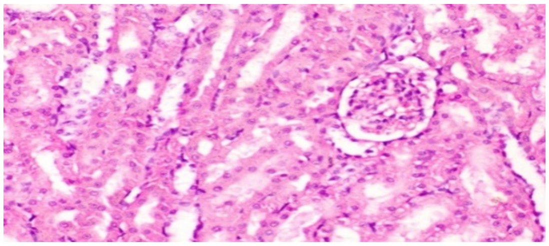


Photo (11): Photomicrograph of rat's kidney from group 3 (fermented milk with 5% honey) (H & E X 400)

Photo (12): Photomicrograph of rat's kidney from group 4 (fermented milk with 1% garlic) (H & E X 400).



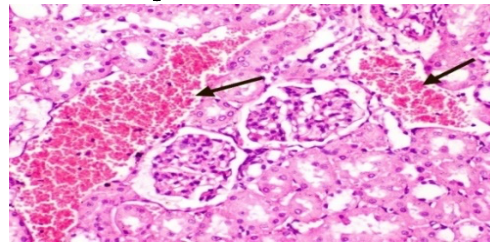
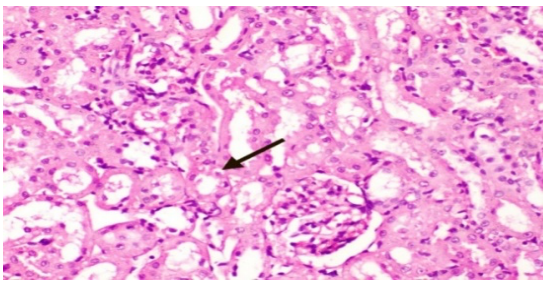


Photo (13): Photomicrograph of rat's kidney from group 5 (fermented milk with 1% ginseng) (H & E X 400).

Photo (14): Photomicrograph of rat's kidney from group 6 (fermented milk with 1% cod liver oil) (H & E X 400).



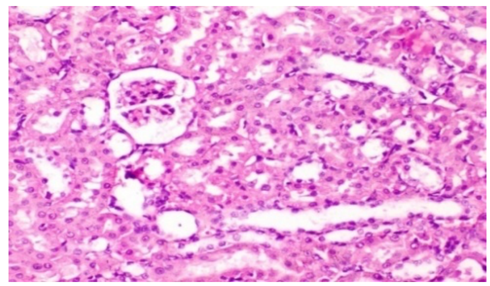


Photo (15): Photomicrograph of rat's kidney from group 7 (fermented milk with 1% chicory) (H & E X 400).

Photo (16): Photomicrograph of rat's kidney from group 8 (fermented milk with 1% mixture of honey and other materials) (H & E X 400).

4- Conclusion

According to this study, fermented milk containing sources of probiotics may shield rats against pb toxicity. The supplements may help restore hematological dysfunctions in rats, reduce tissue Pb buildup, and lessen tissue oxidative stress. Therefore, probiotic supplementation may be viewed as a novel dietary treatment approach in conjunction with traditional chelation, antioxidant, anti-inflammatory, and other supportive therapies for humans to combat pb toxicity.

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التغذية وعلوم الاطعمة

الفئران المصابة بتسمم الرصاص: تأثير الحليب المتخمر المعزز ببعض مصادر البريبايوتيك على المؤشرات البيولوجية و البيوكيميائية

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الملخص العربي:
يمكن للتغيرات في الكائنات الحية الدقيقة في الأمعاء أن تؤثر على المسارات المرتبطة بعوامل الخطر الأيضية للأمراض. لذا تهدف الدراسة الحالية إلى دراسة تأثير الحليب المتخمر مع مكملات البريبايوتيكس على معالجة التغيرات في المعاملات البيولوجية و البيوكيميائية في الفئران المعاملة بخلات الرصاص. تم تقسيم ثمانية وأربعين فأرًا ألبينو ذكرًا، وزن كل منها 110 ± 10 جرام، إلى مجموعتين رئيسيتين. تم استخدام المجموعة الأولى كمجموعة ضابطة سالبة، وتم إعطاء المجموعة الثانية 1 جم / لتر من خلات الرصاص في مياه الشرب لمدة ثلاثين يومًا لإصابتها بتسمم الرصاص. ثم تم تقسيم المجموعة الثانية إلى سبع مجموعات فرعية. تم الاحتفاظ بواحدة كمجموعة ضابطة موجبة ، بينما تم إعطاء الستة الآخرين(مج 8,7,6,5,4,3)الحليب المتخمر مع أنواع مختلفة من البريبايوتكس (5٪ عسل، 1٪ ثوم، 1٪ جينسنغ، 1٪ زيت كبد الحوت، 1% هندباء، و1% خليط البريبايوتكس). تم تحديد المعاملات البيوكيميائية والبيولوجية للمجموعات التي تم اختبارها. أدى استخدام الرصاص لعلاج الحيوانات إلى انخفاض وزن الجسم، والمأخوذ الغذائي، ونسبة كفاءة الوجبة، والهيموجلوبين، والحديد في الدم، وHDL-c، في حين ارتفع وزن الأعضاء النسبي، وإنزيمات الكبد، ووظائف الكلى، ومستوى الدهون. وقد دعم الفحص النسيجي لأنسجة الكبد والكلى النتائج البيوكيميائية. ساعدت مكملات الحليب المخمر التي تحتوي على البريبايوتك على تخفيف التشوهات البيولوجية والبيوكيميائية لدى الفئران المصابة بتسمم الرصاص. لذلك، يمكننا أن نستنتج أن الحليب المتخمر الذي يحتوي على مصادر البريبايوتك، أو خليطها، قد يقلل من عامل خطر التسمم بالرصاص.

الكلمات المفتاحية: الفئران، الرصاص، البريبايوتكس، الحليب المتخمر.