Original Article

Influence Of Cheese Containing Low-Esterified Beet Pectin On Heavy Metals Accumulation In Rats' Tissues

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Abstract

Nowadays, pectin used for removing heavy metals from different biological systems, human and animals' organisms. The daily intake of pectin could be useful against heavy metals exposure of humans and animals. An in vivo experiment on 120 rats was carried out to determine the influence of cheese containing low-esterified beet pectin on heavy metals accumulation in rats' organs, muscles, feces and femur. Lead nitrate and cadmium chloride were used as sources of heavy metals. The concentration of lead and cadmium in feces was higher in all groups receiving heavy metals, compared to control group. The content of heavy metals in fecal samples of groups receiving dairy products was higher compared to the group receiving only heavy metals solution, which shows the possibility of the studied cheese with pectin to influence and contribute to the elimination of heavy metals from the body. The results of this research work showed the effectiveness of consumption low-esterified pectin containing milk products in reducing the quantity of accumulated heavy metals in rats' tissues. In perspective, a new direction of functional food products with detoxifying properties could be developed.

Index Terms— Heavy metals, toxicity, low-esterified beet pectin, accumulation, cheese.

INTRODUCTION

Nowadays, different health problems, serious diseases and human deaths directly related to environmental pollution by numerous contaminants [1,2]. Pollutants' accumulation in organism can take place by concentration in the food chain, inhalation and dermal contact [3]. The most dangerous and common heavy metals are lead, cadmium, arsenic and mercury because of their toxicity [4,5].

Heavy metals cannot be metabolized and completely immobilized, also can exert toxic effect several times, as a result slowly developing and non-communicable chronic diseases can occur, apoptosis and carcinogenesis [6]. Various deleterious effects on renal, hematopoietic, reproductive functions and central nervous system are registered during lead (Pb) poising [4]. Cadmium (Cd) toxicity contributes to heart disease, cancer and diabetes [7,8,9]. Several studies showed that the exposure of pregnant rats to cadmium has an important effect on spermatogenesis, steroidogenesis, and fertility potential of progeny [10].

Furthermore, the toxic effect of heavy metals depends from the various factors such as temperature mechanism of absorption, dose, contact pattern, chemical species, age, sex, genetics and nutritional status [11]. Therefore, human and animals' organisms have toxins chelation and removal systems based metal-binding proteins which allow the body to self-repair and their excretion [12]. However, due to the serious ecological problems, several chelating agents may be helpful for organism. Traditional chelating agents are used in high intoxication cases, but they do not suitable for ongoing use and have side effects as an excretion of essential minerals, even death [13].

Nowadays, different cheap and abundant biosorbents are available, for example, pectin, algal polysaccharides alginate and other natural polymers [14-15]. According to the available literature data, the daily intake of pectin could decrease and influence to the accumulation of heavy metals in the living organisms [16]. Several research studies determined that the pectin with a low degree of esterification and low molecular weight is very effective against heavy metal toxicity and can have positive effect on gut microbiota and health [17,18]. After being absorbed pectins are transported, modified, bounded with metals and forming insoluble pectinates inside the organism, and in the end are excreted by urine and feces [19,20].

By adding pectin into daily food products, as cheese there is a possibility to make pectin's intake more admissible, to solve heavy metals accumulation problem by ongoing use and to improve the population health status [21,22].

The aim of this study was to determine the effect of fermented dairy products, containing low-esterified beet pectin, on heavy metals accumulation in rats' tissues. Such experiments could be an important step for obtaining preliminary data on the possibility of using dairy products containing low-esterified beet pectin to detoxify the human body from contaminants in the future.

MATERIALS AND METHODS

Preclinical studies on the laboratory animals were performed in accordance with the regulatory documents in force in Kazakhstan and with the approval of the local ethical Commission of the S.D.Asfendiyarov Kazakh National Medical University (№5/111, 2021) and on the basis of standard operating procedures adopted by the organization, which comply with the rules adopted by the European Convention for the Protection of Vertebrates Used for research and other scientific purposes.

Experimental animals and design

The experiments were carried out on 120 mature white male laboratory rats weighing 140-160 g, the animals were taken from the vivarium of the NAO "Kazakh National Medical University named after S.D.Asfendiyarov", Almaty, Kazakhstan. The animals were well maintained in special separate boxes inside the specialized vivarium for laboratory animals, under 23-25°C temperature, 60-65% humidity and 12 hours light-dark cycle.

To conduct this experiment, the cheese have been prepared in the scientific and educational laboratory of pharmaceutical products in NAO "Kazakh National Medical University named after S.D.Asfendiyarov" from cow's milk with the addition of low-esterified beet pectin in different amounts: 1.Cheese with 1% of pectin; 2.Cheese with 0.75% of pectin [22].

These products were stored at +4 +6°C, weighed as needed on laboratory scales for animal feeding. Once a day, 5 g of each product was provided per animal.

Lead nitrate and cadmium chloride were used as sources of heavy metals. A solution of heavy metals (50 mg/kg) was prepared.

The experiment duration was 21 days. The animals were divided randomly into the following groups (twenty rats in each group):

Group 1 (control group) – animals of this group fed a standard feed without the heavy metals.

Group 2 (HM (heavy metals) + cheese 1%) – animals of this group received 1 ml of water with lead and cadmium (50 mg / kg) + 5 g of cheese containing 1% pectin.

Group 3 (HM + cheese 0.75%) – animals of this group received 1 ml of water with lead and cadmium (50 mg / kg) + 5 g of cheese containing 0.75% pectin.

Group 4 (HM + standard feed) - animals of this group received 1 ml of water with lead and cadmium (50 mg/kg) + standard feed.

Oral gavage needle with round end was used for administration drinking water with heavy metals for a period of 21 days. The animals were kept on certified compound feeds in accordance with current regulations with free access to water and food throughout the experiment. One hour after the taking water containing heavy metals, pectin-containing products were provided for animals in order to avoid interaction with metals. After that, standard food was provided to the animals during the day. Heavy metals and pectin-containing products were introduced at a fixed time of day in order to prevent errors associated with circadian rhythms.

At the end of the experiment, after ether anesthesia and decapitation, internal organs (liver, kidneys, and heart), muscles and femur were taken for further analysis. Fecal sampling was also carried out every 7 days. For the further analysis all tissues samples of each group were homogenized and stored at -20℃ until analysis.

RESULTS

During the entire period of the experiment, every day according to the plan of scientific work, laboratory rats, depending from the group, received solution of heavy metals and/or fermented milk products with pectin, and with standard feed. The concentration of lead in control feed was 0.05 ± 0.02 Pb kg^{-1}.

The concentration of lead and cadmium in feces was higher in all groups receiving a solution of heavy metals, compared to control group (Tables 1-2). Which in turn indicates the continuous excretion of metals through the digestive tract.

Table 1. The amount of cadmium in fecal samples of the studied groups, µg/l

Groups	1 week	2 week	3 week
Group 1 – Control group	0,62±0.06	0,53±0.04	0,5±0.04
Group 2 – HM + cheese 1%	0,54±0.03	23,9±1.0	24,7±1.5
Group 3 – HM+ cheese 0.75%	0,23±0.04	20,2±1.2	22,2±1.3
Group 4 - HM + regular feed	0,65±0.06	8,0±0.8	8,5±1.1

* Statistically different from the control group (P<0.001)

Table 2. Amount of lead in fecal samples of the studied groups, µg/l

Groups	1 week	2 week	3 week
Group 1 – Control group	0,52±0.05	0,63±0.04	0,4±0.05
Group 2 – HM + cheese 1%	0,64±0.03	20,2±1.0	22,7±1.6
Group 3 – HM+ cheese 0.75%	0,33±0.02	18,2±1.2	19,5±1.2
Group 4 - HM + regular feed	0,75±0.03	8,4±2.3	8,7±1.2

* Statistically different from the control group (P<0.001)

In the Group 4, the cadmium content increased at the end of the second week (8.0±0.8) of the experiment compared to the first week (0.65±0.06), by the end of the third week (8.5±1.1). The content of cadmium and lead in fecal samples of groups receiving dairy products with the addition of low-esterified beet pectin was higher compared to group 4, which shows the possibility of the studied pectin to influence and contribute to the elimination of heavy metals from the body. The highest lead and cadmium content were in the samples of the Group 2 and at the end of the experiment was 22.7±1.6 µg/l and 23.9±1.0µg/l, respectively.

At the end of the three-week experiment, in the groups receiving a solution of heavy metals, the metal content in the internal organs, muscles and femur was higher compared to the control group. The highest content of lead and cadmium in all the samples studied was in Group 4. This group received a solution of heavy metals with a standard feed (Table 3-4).

Table 3. The amount of cadmium in the internal organs, muscles and femur, µg/l.

Groups	Liver	Kidney	Heart	Muscle	Femur
Group 1 – Control group	11,7±0.5	13,95±0.5	10,2±1.3	9,7±4.5	8,6±1.2
Group 2 – HM + cheese 1%	150,2±0.8	111,8±1.2	119,3±6.3	112±5.2	70,6±1.2
Group 3 – HM+ cheese 0.75%	164,4±2.2	129,9±2.1	131,5±7.1	130,1±5.4	81±1.2
Group 4 - HM + regular feed	277,8±0.7	184,2±1.5	180,5±8.1	196,4±5.2	113,8±1.2

* Statistically different from the control group (P<0.001)

Table 4. The amount of lead in the internal organs, muscles and femur, µg/l.

Groups	Liver	Kidney	Heart	Muscle	Femur
Group 1 – Control group	17,3±1.2	1,9±1.2	17,6±1.2	2,0±1.2	18,7±1.2
Group 2 – HM + cheese 1%	140,1±7.2	102,5±1.2	39,3±1.2	45,8±3.4	85,5±1.2
Group 3 – HM+ cheese 0.75%	152,0±6.3	111,2±1.3	45,5±0,8	59,9±2.2	91,3±1.2
Group 4 - HM + regular feed	286,5±1.2	219,5±1.2	110,5±1.2	91,2±5.7	315,6±5.2

* Statistically different from the control group (P<0.001)

The introduction of dairy products containing pectin led to significant changes in the lead content in the internal organs, muscles and femur, and contributed to a decrease in this indicator in Groups 2-3 compared with Group 4. In the Group 2, which received the solution of heavy metals and cheese with a content of 1% pectin, the amount of lead and cadmium in the internal organs and femur was more pronounced. The highest amount of cadmium was contained in liver of all studied groups. In the liver, the content of lead and cadmium was reduced when cheese containing 1% pectin was included in the diet of rats. For example, in the samples of Group 4, the cadmium content was 277.8±0.7 μg/l and lead 219.5±1.2 μg/l, in Group 2, 150.2± 0.8 and 102.5±1.2, respectively.

The quantity of heavy metals in samples of Groups 2-3 compared to Group 4, was 1.5-2 times lower.

In the groups receiving pectin-containing products the lead content in femoral samples significantly was lower (85.5±1.2), compared with Group 4, where the lead content in femoral samples was 315.6±1.2 μg/l (Table 4).

DISCUSSION

According to the literature data, after cadmium uptake and passing into blood it is head directly to the liver, then through the blood enter into the biliary tract and after enzymatic degradation reaches the small intestines [8]. Several authors showed that the main internal organ of cadmium concentration was a liver [7,9]. Data of this study confirm the results of previous mentioned studies. Some literature data mention kidney as the main organ for long-term cadmium accumulation [7]. The experiment results which was carried out showed that the concentration of cadmium in the kidney samples was high, but lower than in liver samples. Such results could be related to the experiment duration, in the case of long period experiment results might be different. Research results of long-term oral administration of Cd showed that tissue accumulation does occur both in the liver and kidney [23].

After lead uptake, according to the available literature data, via the gastrointestinal track it is absorbed into the bloodstream and head directly to the different organs where could be accumulated. The experiment on female Wistar rats of different ages showed that the main matrix for Pb accumulation was the bone, then comes liver, kidney, muscle, brain [4]. According to the results of our experiment, complimentary the highest concentration of lead was determined in the femur samples, then in liver and kidney, the lowest concentration was determined in heart and muscle. Unfortunately, brain samples were not taken for the analysis.

Excretion of heavy metal takes place via feces and urine [24]. Zhao et al. (2008) showed the effectiveness of modified citrus pectin in detoxification of children organisms with a high level of lead in the blood, after pectin consumption in 24 h heavy metal concentration decreased in the blood and increased in the urine. The one of the advantages of such treatment that during detoxification the release of essential minerals does not occur [11]. In other literature, the effectiveness of the commercial pectin supplement was showed in the urinary excretion of arsenic, cadmium and lead [25]. Therefore, the concentration of heavy metals was not determined during this study, only feces samples were taken for analysis. The results show that lead and cadmium were partly eliminated by feces, and the highest elimination rate was in the groups received cheese containing 0.75-1% of low-esterified beet pectin. Further more detailed studies need to be done.

CONCLUSION

To clarify toxicity of main heavy metals and understand their distribution in human tissues and organs – *in vitro* and *in vivo* animal studies is still important. The results of the analyses obtained during this research work showed the effectiveness of the use of low-esterified pectin in reducing the amount of heavy metals in the internal organs, muscles and femur. The minimum content of heavy metals was observed in the samples of the group that received a solution of heavy metals and cheese with a content of 1% low-esterified pectin. Based on the use of the high complexing ability of low-esterified pectin, which have the properties of removing toxic substances, a new direction for the creation of functional food products with detoxifying properties will be developed.

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