Journal of Medical Sciences

ISSN 1682-4474

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Research Paper

J. Med. Sci., 13 (8): 647-656
15th November, 2013
DOI: 10.3923/jms.2013.647.656

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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Comparative Lead-removing Activity of the Non-starch Polysaccharides

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Lead is a ubiquitous environmental pollutant that poses a serious threat to human health. Therefore effective methods diminishing negative influence of this metal are of great importance. In this study lead binding activity of such non-starch polysaccharides as high and low esterified pectin, calcium alginate and lignin as well as of activated charcoal was estimated under *in vitro* and *in vivo* conditions. The adsorption of lead ions from aqueous solution by mentioned substances was studied in a batch sorption system. The lead-binding capacity of all polysaccharide compounds was highest within the pH range 4.0-8.0. The Langmuir and Freundlich sorption models were applied to describe the isotherms and sorption constants. Interactions between non-starch polysaccharides except lignin could be well interpreted by the Langmuir model. In experiments estimating lead removal from inner organs and femur in rats preliminary given the heavy metal solution for three weeks, calcium alginate and low esterified pectin were the most effective agents studied compared with others. They contributed to reduced lead concentration in organs and femur as well as increased metal content in feces of laboratory animals. The results suggest that low esterified pectin and calcium alginate may be considered perspective compounds purposed for prevention and treatment of chronic lead poisoning.

Key words: Heavy metals, lead, non-starch polysaccharides, equilibrium study, rats

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INTRODUCTION

Fast growth of the industrial activity and technological development during the recent years resulted in vast release of the metal pollutants into the environment. One of the most common pollutants is lead. Accumulation of this metal in the food chains and persistence of the lead ions in the ecosystems poses a serious danger to the public health due to its pronounced toxicity (Markowitz, 2000). In the USA more than one million construction workers are exposed to excessive amount of lead and other heavy metals (Levin and Goldberg, 2000). Approximately 500,000 american children aged from 1 to 5 year were found to have elevated blood lead level. This is considered as greater concern due to high vulnerability of children to lead exposure even in small concentration (Rogan and Ware, 2003). The main sources of lead ions posing a risk to humans are diet, lead-based paint, metal ions in soil and dust around industrial areas and continuing use of leaded gasoline in some countries (Paoliello and De Capitani, 2005; Vaird *et al*., 2004).

When entered the human body lead acts as a potential neurotoxin (Lindsky and Schneider, 2003) with no beneficial effects. In inner media lead ions binds proteins containing thiol groups as well as amide, phosphate and carboxyl ones. The largest amounts of the lead ions are stored in bones. Besides, lead can be found in brain, kidney and liver. Therefore, chronic exposure to lead inevitably affects nervous, immune and hematopoietic systems, as well as kidneys and gastrointestinal tract (Mazzolini *et al*., 2001; El-Safty *et al*., 2004). Nowadays the therapy of acute and chronic lead poisoning includes the following measures: elimination of all lead sources, administration of calcium and calcium containing products, nutritional and vitamin support and the use of specific metal chelators. Chelators are the agents possessing a unique capacity to irreversibly bind metal ions and eliminate them from the body through intestine and kidney (Kalia and Flora, 2005). Unfortunately up-to-date chelators cannot be used for a long treatment of the chronic lead poisoning due to their toxic effects on various organs and systems as well as on mineral balance (Smith *et al*., 2000). Therefore, safe and effective agents with marked metal binding activity are required for treatment as well as for prevention of the chronic lead poisonings.

Today among the most perspective compounds for removal of the heavy metals are activated carbons and dietary fibers such as lignin, alginates and pectins possessing considerable metal binding capacity. All of them except carbons belong to a group of substances termed non-starch polysaccharides which were found to be effective for the wastewater purification (Serguschenko *et al*., 2007). Non-starch polysaccharides are considered attractive because they possess a capability of lowering transition metal-ion concentration to parts per billion level, they are widely available, environmentally safe and suitable for human consumption (Schmubil *et al*., 2001; Khotimchenko *et al*., 2007). Moreover, dietary fibers are considered as the essential components of the human diet exerting numerous beneficial effects on all systems and organs in the human body.

In the present work activated charcoal and non-starch polysaccharides such as lignin, calcium alginate and low and high esterified pectins were investigated regarding their metal binding capacity. In the first part of experiment the equilibrium studies were carried out focused on the elucidation of basic physicochemical activity of these compounds. In the final part of the work efficacy of the compounds was tested using the laboratory rats with modeled lead intoxication. The main goal of this study was to determinate the relationship between physicochemical activity of the lead-binding compounds and their effects on lead removal in laboratory rats.

MATERIALS AND METHODS

Chemicals: Pure lead acetate was purchased from Sigma Chemical (St. Louis, Mo). All other chemicals were of the highest quality available. Distilled water was used throughout.

High-esterified citrus pectin without additives was obtained from Herbsrtaith and Fox, Germany. The stated degree of esterification of this preparation was 60.0%. The pectin preparation contained no acetyl or amide groups. Initially pectin with a degree of esterification of approximately 1.2% was prepared. During this process 100 g of high esterified citrus pectin was de-esterified in 1600 mL 50% ethyl alcohol containing 20 g NaOH and 20 g KOH (30 min at 20°C). After acidification, pectin was isolated from ethanol by filtration.

Sodium alginate (type HV) without additives was obtained from Kelco (California, USA). Calcium alginate was prepared as follows. Sodium alginate (200 g) was suspended in 1000 mL of 70% ethanol. Shaking intensively, 8 g of $CaCl_2$ $6H_2O$ diluted preliminary in 100 mL of 70% ethanol were added. Calcium alginate obtained was separated with a porous glass filter with a mesh of 40 mkm, rinsed with 500 mL of 70% ethanol and dried at 60°C.

Granulated lignin was obtained from Saintec, Russia, under the trade mark Polyphepan, which is approved as the agent for therapy of chronic and acute heavy metal poisoning. Tablets of activated charcoal were obtained from Irbit, Russia. The tablets were ground to a powder before being used in experiment.

Sample analysis: The galacturonan content of the pectin and alginate preparations was determined colorimetrically by the m-hydroxydiphenyl method (Blumenkratz and Asboe-Hansen, 1973). The degree of esterification was characterized using titrimetric analysis with 1 M NaOH solution in 50% ethanol in the presence of Hintone indicator (Afanas'ev *et al.*, 1984). Intrinsic viscosity of low esterified pectin was determined in 0.05 M NaCl/0.005 M Na-oxalate at 25.0°C and pH 6.0 using an Ubbelohde viscosimeter. The intrinsic viscosity was related empirically to the molecular weight by the Mark-Howink equation (Kravtchenko and Pilnik, 1990) presented as $\mu = KM\alpha$ that is generally used for definition of the pectin molecular weight using values for the constants α (0.79) and K ($216 \cdot 10^{-6}$) that are suitable for pectins and alginates (Grassi *et al.*, 1996). The calcium content in the calcium alginate sample was assayed by atomic absorption and expressed in mg g^{-1} of the sample (Kostecka, 2000).

Experimental procedures: Lead stock solutions with concentration 20.6 g L^{-1} (0.1 M) were prepared using analytical-reagent grade $PbNO_3$. The stock solution was then diluted to give standard solutions of appropriate concentrations with controlled pH at 6.0 achieved by addition of either 0.1 M HCl or 0.1 M NaOH. Batch sorption experiments were conducted in 20 mL beakers and equilibrated using a magnetic stirrer. Then 1.0 mL aliquots of these standard solutions were placed in 20 mL beakers with 10 mL of solution containing 0.05 g of dry preparations. Then the total volume of the solution prepared was made up to 20 mL by addition of distilled water. Removal of the compounds studied from the lead solution was performed by the use of centrifugal force unit 3000 g for 10-20 min with the following filtration through a glass filter with a pore size 100-120 μm. Concentration of lead ions in the supernatant obtained was analyzed using an atomic absorption spectrophotometry method. The effect of lead sorption was studied in a pH range 2.0-10.0. The pH of the initial solution was adjusted to the required pH value using either 0.1 M HCl or 0.1 M NaOH. Compounds were equilibrated at the particular pH for about 120 min at 400 rpm and at initial lead concentration of 0.6 $g \times L^{-1}$ using a bath controlled at 24°C. Each experiment was triplicated under identical conditions. A negative control experiment with no polysaccharide added was simultaneously carried out to ensure that the lead removal was caused by the polysaccharide binding activity and not by the beaker or filter influence. The parameters obtained were subjected to a one-way analysis of variance using a software package SPSS (Statistical Package for Social Sciences) for Windows, version 11.0 with a confidence level of 95% ($p<0.05$).

The effect of agitation period was also studied to determine the optimum conditions for sorption of the lead ions. For batch kinetic studies 10 mL of solution containing 0.05 g of dry compound were equilibrated at optimum condition as mentioned earlier. The sorption system was placed in 20 mL beakers and stirred by a magnetic stirrer. At present time intervals, the aqueous samples (5 cm^3) were taken and the lead concentration was assessed.

Sorption equilibrium studies were conducted at optimum condition using a contact time of 120 min at pH 2.0-10.0. According to the preliminary results pH value 6.0 was considered as most acceptable because all polysaccharides at this point possess highest binding activity and pH control requires minimum amounts of HCl and NaOH. Bath controlled temperature was 24°C. Isotherm studies were conducted with a constant pectin preparation amount (0.05 g) and varying initial concentration of lead ions in the range of 0.05- 0.6 $g \times L^{-1}$. Each experiment was at least duplicated under identical conditions.

The metal accumulation (q) was determined as follows:

$$q_e = \frac{(C_0 - C_e) \cdot V}{W} \qquad (1)$$

where, C_0 is the initial lead concentration ($mg \times L^{-1}$), C_e is the final or equilibrium lead concentration ($mg \times L^{-1}$), V is the volume of the lead solution (mL) and W is the weight of the dry samples studied (g).

The amount of the metal ions bound by the compounds was expressed in mg per g of the dry sorbent.

Animals and diet: Male Wistar rats were obtained from Institute of Marine Biology (Vladivostok, Russia). The rats weighing 130 to 160 g were housed in stainless steel wired cages (in groups of four per cage) and kept in an isolated room at a controlled temperature 20-22°C and ambient humidity 60 to 65%. Lights were maintained on an artificial 12 h light-dark cycle. Animals were first adapted to the facility for 1 week and provided with water and standard feed ad libitum. The composition of the standard diet was as follows (g 100 g^{-1}): casein, 21.0; cellulose, 5.3;

sunflower oil, 7.0; cholesterol, 1.0; sucrose, 15.0; starch, 45.9; methionine, 0.3; minerals, 3.5 and vitamin mixture, 1.0. All animal experiments were conducted in accordance with the guide for the care and use of laboratory animals of Far Eastern Federal University, which is following the Guiding Principles in the Use of Animals in Toxicology.

Experimental design: This part of study consisted of the two experiments estimating influence of pectins, calcium alginate, lignin and activated charcoal on the removal of lead from inner organs and bones and fecal lead elimination.

In the first experiment after the adaptation period, 80 rats were randomly divided into seven groups. All animals except the Control group were daily given 1 mL of lead acetate solution containing 50 mg of lead per kg through gastric gavage for three weeks. The control group received 1 mL of distilled water through gastric gavage. Then half of the control rats and animals of the test group were killed by decapitation under slight anesthesia; inner organs and femora were removed, weighed, rinsed and stored until analysis. For the next 21 days the remaining rats of "Control" and "Lead" groups were given only standard diet whereas rats of other groups were daily administered suspensions containing 0.5 mg kg^{-1} polysaccharides samples or activated charcoal 1 h before feeding through gastric gavage. At the end of experiment rats were killed and inner organs and femora were removed, weighed, rinsed and prepared for lead analysis.

In the second experiment rats were randomized into seven groups. All animals except the Control group groups were daily given lead acetate as described before for three weeks. The control group received daily 1 mL of distilled water. Then administration of lead was discontinued and all rats were put into individual cages. For the next 28 days all groups were fed standard diet and at the same time rats of the test groups were additionally given 1 h before feeding through gastric gavage water suspensions containing 0.5 g per kg of the pectin samples, calcium alginate, lignin and activated charcoal. Feces were collected daily, dried, ground and prepared for the lead analysis.

Metal concentration analysis: Lead concentration in solution filtered in a course of the in vitro studies on lead binding capacity of polysaccharides and activated charcoal was assessed using a titrimetric method with EDTA. The lead content in heart, kidney, liver and femur removed from the rats as well as in feces was estimated by atomic absorption spectrometry (Parsons and Slavin 1993).

Statistical analyses: Findings indicating the lead contents in organs and bones are presented as Mean±SEM. Results obtained at the end of the study were analyzed using one-way Analysis of Variance (ANOVA) and post hoc Tukey’s test. Differences with a value of $p<0.05$ were considered statistically significant.

RESULTS

Polysaccharide characteristics: Chemical analysis of the pectin samples showed the following results. The galacturonic acid concentration of both samples was 78.0%. The assay showed degree of esterification of the high esterified pectin to be approximately 60.8%. Degree of esterfication of the low esterified pectin was 1.2%. The free and esterified carboxyl groups in the pectin macromolecules were distributed in a random pattern. The intrinsic viscosity of high-esterified pectin used was 352 mL g^{-1} of galacturonan. Molecular weight calculated using Mark-Howink equation was around 225 kDa. The intrinsic viscosity of the low esterified pectin sample was 201 mL g^{-1} of galacturonan and calculated molecular weight was approximately 20 kDa. Both samples were soluble in water.

The uronic acid content of the calcium alginate sample was figured out to be 77.3%. The intrinsic viscosity of original sodium alginate used for preparation of calcium alginate was 1,270 mL g^{-1}. Calculated molecular weight of the original sodium alginate and calcium alginate used in experiments was $403{\cdot}10^3$ Da. The calcium content of the calcium alginate sample was 7.25%. This indicates 82.5% of carboxyl groups in preparation are presented in calcified form, which means calcium content in the sample is 38 mg g^{-1} of the polysaccharide. Calcium alginate beads were not soluble in water.

***In-vitro* lead-binding activity:** Figure 1 shows the effects of agitation period on the metal uptake of the non-starch polysaccharides and activated carbon samples. The time required to reach equilibrium can be found with different lead uptake values obtained after various batch sorption periods. The amount of lead bound by all samples increases within the beginning of agitation period and attains equilibrium in about 60 min. The main differences in the sorption rates between these compounds were observed within the first minutes of the agitation period. Water soluble pectin samples bound more than 80% of their highest lead uptake in less than 5 min of agitation. At the same time during the first five minutes of the agitation period insoluble calcium alginate bound about 50% of their highest uptake under given conditions. Interaction of the lignin with the metal ions was the

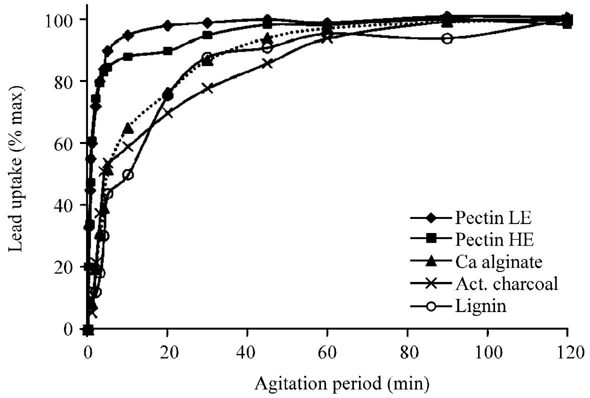

Fig. 1: Effect of the agitation period on the lead uptake by non-starch polysaccharides and activated charcoal

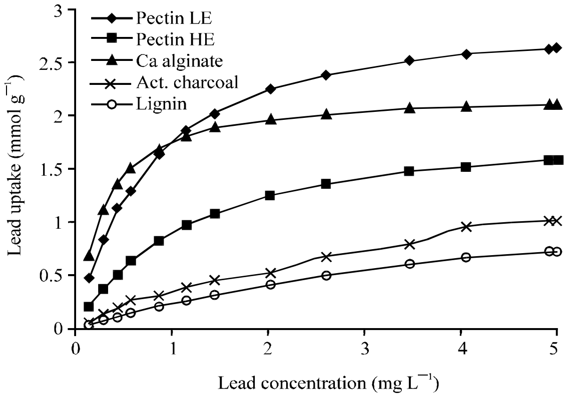

Fig. 3: Equilibrium sorption of lead ions from aqueous solution by non-starch polysaccharides and activated charcoal

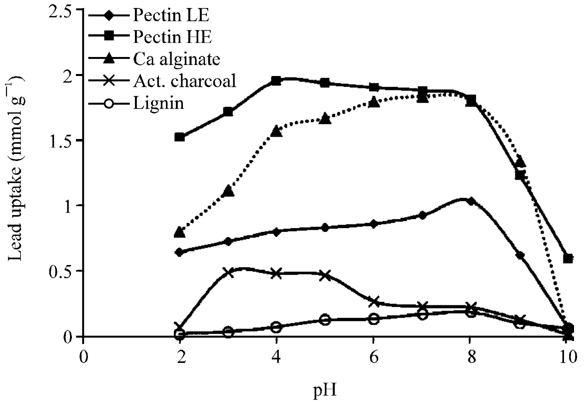

Fig. 2: Effects of pH on the lead uptake by non-starch polysaccharides and activated charcoal

slowest and it bound about 40% of its maximum sorption capacity within first 5 min of agitation. After five minutes of agitation the amount lead bound by all substances gradually increased and in 60 min their values reached 100% of the highest lead binding capacity and the differences between all samples was insignificant. The longer period required for insoluble samples to bind lead ions is probably dependent on the rate of metal diffusion into insoluble beads.

The pH of solution strongly affects the sorption capacity of any binding material interacting with metal ions. Therefore, the measurement of the sorption capacity at various pH means estimation of selectivity of polysaccharide compounds to protons in comparison to that of lead ions. Figure 2 illustrates the effects of pH on the lead uptake by the materials studied. The highest lead binding capacity of the both pectins and sodium alginate was found within the pH range of between 4.0 and 8.0 whereas it is for lignin was found within pH 3.0-5.0. Lead binding capacity of activated charcoal did not significantly changed at various pH. At pH values higher than 8.0 there was a dramatic decrease of the uptake capacity of all compounds, because polysaccharides become unstable (Lofgren *et al*., 2002) and the lead ions form insoluble hydroxide which barely be bound via ion exchange mechanism. Below pH 8.0 sorption gradually increases with the rise of pH. This could be explained by the phenomenon that the lower the pH, the more protons are available to carboxyl groups reducing the number of binding sites in the polysaccharide or carbon molecule.

Removal of lead by non-starch polysaccharides and activated charcoal as a function of final metal concentration was studied at pH 6.0. Since the data for the curve are obtained at the same temperature, the curve is an isotherm. Therefore, there is a defined distribution of the metal ions in the sorption system, which can be expressed by one or more isotherms (Findon *et al*., 1993). Figure 3 shows the sorption curves displaying the amount of lead ions bound by the all samples used in the study. It can be seen that metal uptake increases while final metal concentration becomes higher. The isotherms are characterized by the initial region, which is represented as being concave to the concentration axis.

The isotherm reaches a plateau, which can typically be described by the one or more mathematical models. The results obtained in our study were analyzed using Langmuir and Freundlich adsorption isotherms. The Langmuir equation is often used to describe equilibrium sorption isotherm, which is valid for monolayer sorption with a finite number of identical sites and is given by:

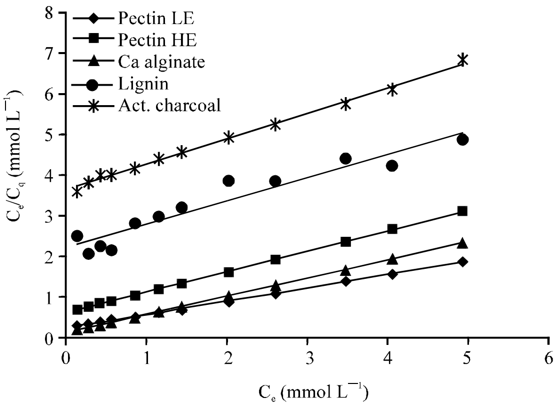

Fig. 4: Langmuir plot for the sorption of lead ions by non-starch polysaccharides and activated charcoal

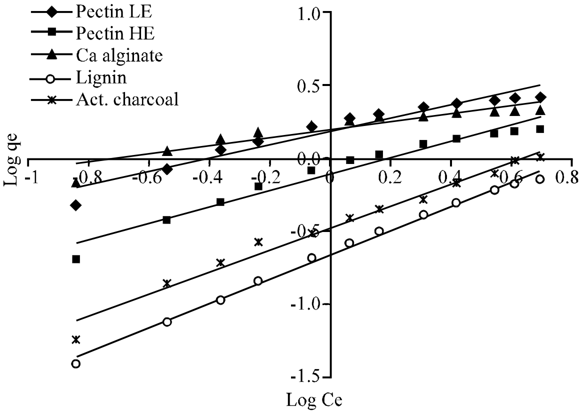

Fig. 5: Freundlich plot for the sorption of lead ions by non-starch polysaccharides and activated charcoal

$$q = (q_{max}bC_e)/(1+bC_e) \quad (2)$$

where, q_{max} is the maximum sorption at monolayer (mg g^{-1}), C_e a final equilibrium concentration of lead ions, q the amount of lead ions bound per unit weight of the polysaccharide compound at final equilibrium concentration (mg g^{-1}) and b is the Langmuir constant related to the affinity of binding sites (mL mg^{-1}) and is considered as a measure of the energy of sorption.

The following linearized plot of the Langmuir equation was used in this study which gives q_{max} and b:

$$C_e/q_e = (C_e/q_{max})+1/(q_{max}b) \quad (3)$$

The widely used empirical Freundlich equation based on sorption on a heterogeneous surface is given by:

$$\log q_e = \log K_F + \frac{1}{n}\log C_e \quad (4)$$

where, K_F and n are Freundlich constants indicating sorption capacity (mg g^{-1}) and intensity, respectively. K_F and n can be determined from linear plot of log q_e against log C_e.

Calculated results of the Langmuir and Freundlich isotherms are given in the Table 1. The results show that sorption of lead by low and high esterified pectins, calcium alginate and activated charcoal were better correlated (R^2>0.99) with the Langmuir equation. Interaction of lignin molecules with the lead ions is better described by the Freundlich model (R^2>0.99). It can be explained by the presence of finite number of homogenous binding sites in the pectin and alginate molecules, which is the basic condition of the Langmuir sorption model. Lead ions bound onto the homogenous carbon surface also form monolayer. Perhaps lignin structure is rather heterogenous and one metal ion can interact with different binding sites. Figure 4 shows the Langmuir plot for the sorption of lead by the compounds studied. According to calculated Langmuir parameters highest binding capacity is characteristic of calcium alginate although low esterified pectin possesses a little lower lead binding activity.

Table 1: Langmuir and Freundlich isotherm constants and correlation coefficients of led binding capacity of the non-starch polysaccharides and activated charcoal

	Langmuir			Freundlich		
	b, (mL mg^{-1})	$q_{max,}$ (mg g^{-1})	R^2	$K_{F,}$ (mg g^{-1})	n	R^2
Pectin HE	0.811	1.990	0.9998	0.897	1.781	0.9349
Pectin LE	1.329	3.055	0.9998	1.199	2.178	0.9594
Ca alginate	3.472	2.241	0.9999	1.212	3.594	0.8601
Lignin	0.258	1.750	0.9209	0.517	1.204	0.9951
Activated charcoal	0.171	1.606	0.9958	0.618	1.322	0.9800

The sorption coefficient b that is related to the apparent energy of sorption for calcium alginate was quite greater than that of low esterified pectin.

Figure 5 shows the Freundlich plots of the lead sorption by the non-starch polysaccharides and activated charcoal. According to the values of R^2 this model fits for description of interaction between the lead ions and lignin and activated charcoal. Nevertheless, it can be seen that lignin exerts low lead binding activity.

The mechanism of interaction between lead ions and non-starch polysaccharides is to be elucidated by binding of the metal ions with carboxyl groups located on the polymer molecules and functioning as the binding sites. The results show that intensity of binding processes and sorption capacity does not depend on solubility of the

Table 2: Lead content in inner organs and femur in rats given non-starch polysaccharides and activated charcoal in dose 0.5 g kg^{-1} daily for 21 days after advance administration of lead acetate

	Lead concentration (µg g^{-1} of dry weight)			
Group	Liver	Heart	Kidney	Femur
20 day				
Control	8.3±2.3	8.2±0.8	16.1±2.1	40.9±6.9
Lead acetate	35.7±1.8*	14.8±1.9*	1207.0±256.4*	312.2±40.4*
42 days				
Control	15.1±2.7	11.3±1.2	29.3±3.8	36.1±4.8
Lead acetate	13.3±1.4	14.7±0.9	1034.5±133.7*	296.8±46.5*
Lead acetate+ pectin HE	23.5±3.2*[a]	25.2±0.6*[a]	681.1±116.2*[a]	308.3±32.4*
Leas acetate+ pectin LE*[a]	46.0±14.4*[a]	44.9±9.9*[a]	645.8±122.9*[a]	153.1±13.2
Lead acetate+ Ca alginate	38.5±1.7*[a]	39.7±3.3*[a]	390.2±38.2*[a]	152.7±23.9*
Lead acetate+ lignin	11.6±0.6	15.0±0.9	702.7±138.8*	329.5±34.9*
Lead acetate+ activated charcoal	16.2±1.7	14.7±1.3	820.5±123.8*	332.7±31.4*

*Significant difference ($p<.05$) compared with the "Control" group, [a]Significant difference ($p<.05$) compared with "Lead acetate" group using post hoc Tukey's test

compound but closely relates to the number of the carboxyl groups in its structure (Plazinski, 2011). This implies formation of the junction zones between carboxyl groups of polysaccharides and the lead ions according to the "egg-box" model. Activated charcoal binds lead via physical adsorption of the ions on its surface as it was initially described in the Langmuir model.

These results suggest that under *in vivo* conditions calcium alginates and low esterified pectin will be the most effective agent for removal of the lead ions in rats.

Animal studies: In this study three weeks administration of lead resulted in significant increase of the metal concentration in inner organs and bones. In liver the lead contents were 4.1 times higher than in the "control" group, in heart-52.8% higher, in kidney-75.4 times higher and in femur-7.6 times higher.

Within next 3 weeks the lead concentration did not significantly change in inner organs and femur (Table 2). The results show that administration of the pectins and calcium alginate led to accelerated removal of the lead ions from inner organs. Both high esterified and low esterified pectins as well as calcium alginate contributed to metal elimination from kidney. Administration of the pectin with high degree of esterification resulted in 34.1% reduced lead concentration in kidney whereas administration of the low esterified pectin led to 37.6% lowered lead storage. After the use of calcium alginate, the lead contents in kidney were reduced by 39.7%. Lignin and activated charcoal did not affect lead concentration in kidney. In femur the lead quantity also did not significantly changed as a result of administration of lignin, high esterified pectin and activated charcoal. But low esterified pectin and calcium alginate contributed to almost two-fold reduction of lead content in femur. It should be mentioned that after administration of calcium alginate and low esterified pectin there was significant increase of lead content in liver and heart in comparison with animals of control group and even with animals given lead acetate and not treated with polysaccharides. These findings can be explained by fast removal of lead from bones and kidneys, rapid increase of blood lead level and consequent accumulation of the metal in well perfused organs such as liver and heart. This phenomenon is sometimes called a "bound" effect (Gerhardsson *et al*., 1999). It may present a danger due to high lead toxicity, so fast lead elimination is recommended to be avoided if possible. The doses of any agent proposed for removal of the stored lead should be carefully calculated and not provoke elevation of blood lead level more than 10 µg dL^{-1}, which is a minimal toxic lead concentration (Markowitz, 2000).

In all groups of animals given the lead acetate per os significantly increased metal concentration in feces was registered indicating continuous lead elimination through the digestive tract. It was found that in three weeks lead acetate administration did result in more than 1500 times rise of lead removal through gastrointestinal tract within indicated period. Then removal of lead ions gradually reduced within next four weeks. The use of calcium alginate as well as low esterified pectins contributed to more effective elimination of the lead ions from the body with feces. High esterified pectin also contributed to enhanced lead elimination but it was less effective than low esterified pectin. Administration of lignin and activated charcoal did not change lead concentration in feces within the whole period of experiment (Table 3).

DISCUSSION

Lead is a potential toxin possessing no physiological value. Entered in low doses it affects functions of organs manifesting in lowered intelligence and behavioral problems (Lindsky and Schneider, 2003), impaired immune system (Singh *et al*., 2003), altered growth and structure of bones and tooth dentine (Markowitz and Shen, 2001), induced renal dysfunction (Brewster and Perazella, 2004) and disturbed endocrine system (Baccarelli *et al*., 2000). Unfortunately the medical treatment of patients exposed to lead generally does not result in elimination of the toxicant, in particular, in a case of chronic poisoning. Application of modern chelators is not shown to be safe and is usually associated with some difficulties due to their toxic effects such as dyspeptic signs (Rogan, 2000),

Table 3: Effect of the non-starch polysaccharides and activated charcoal on the lead excretion with feces within 4 weeks in rats preliminary exposed to lead

	Fecal lead concentration ($\mu g\ g^{-1}$)					
Group	Day 0	Day 21	Day 28	Day 35	Day 42	Day 49
Control	3.3±1.1	3.4±0.9	3.7±1.8	3.1±1.2	3.8±1.1	3.6±0.9
Lead acetate	2.9±0.8	5337.5±130.8*	68.9±7.3*	58.5±5.2*	50.6±4.8*	30.0±1.9*
Lead acetate+Pectin HE	3.1±1.0	5427.1±175.8*	79.2±6.0*	77.4±5.3*a	70.8±4.3*a	52.5±2.4*a
Lead acetate+Pectin LE	2.6±0.9	5123.9±186.2*	93.1±6.8*a	99.2±6.0*a	85.9±5.1*a	79.2±3.6*a
Lead acetate+Ca alginate	3.5±0.9	5648.0±202.3*	94.5±6.4*a	97.4±5.9*a	85.2±4.9*a	86.4±2.6*a
Lead acetate+Lignin	2.9±0.8	5290.4±132.9*	65.8±8.5*	55.3±7.8*	52.9±9.7*	34.6±5.4*
Lead acetate+Activated charcoal	2.5±0.7	5233.0±173.8*	66.8±9.6*	59.7±7.9*	51.8±5.8*	31.6±4.7*

*Significant difference ($p<.05$) compared with the "Control" group, aSignificant difference ($p<.05$) compared with "Lead acetate" group using post hoc Tukey's test

allergic reactions (Klaassen, 1996), renal dysfunction (Cranton and Frackelton, 2001) and mineral imbalance manifesting in reduced blood levels of calcium, iron, phosphorus, potassium and magnesium (Smith *et al*., 2000).

It is well known that non-starch polysaccharides are essential food constituents possessing various beneficial effects on human systems with no pronounced adverse effects (Krauss *et al*., 2001). Therefore, their usage for elimination of the heavy metal ions from the body may considered as very prospective because they can be used for the long periods with low risk of adverse reactions.

In our study we have demonstrated that such non-starch polysaccharides as low esterified pectin and calcium alginate possess high metal binding capacity due to their physicochemical properties. According to the "egg-box" model of interaction between polysaccharides and bivalent metals (Plazinski, 2011), the quantity of the metal bound to the polysaccharide is determined by the number of active carboxyl groups. In accordance to this model in our experiments pectin with the degree of esterification about 1.2% exerted higher lead binding activity in comparison to high esterified pectin. High esterified pectin is characterized by a major part of carboxyl group to be occupied with methyl radicals preventing interaction with the metal ions. Structural characteristics of the alginate molecule provide all carboxyl groups to be in active state. Therefore, calcium alginate also effectively binds lead ions. Lignin and activated charcoal have another mechanism of interaction with metals generally caused by physical adsorption. This mechanism is less effective than "egg-box" complex-formation. Thus, these substances did not effectively remove heavy metal ions from inner organs.

It should be noted that as a majority of polysaccharides, pectins and alginates are heterogeneous compounds regarding their structure, molecular weight and other physicochemical properties. These parameters of non-starch polysaccharides vary from one species to another and also during the different developmental stages (Chang *et al*., 1994) as well as in the process of chemical and enzymatic modifications (Hotchkiss *et al*., 2002). Therefore, in experiments estimating pharmacologic efficiency of different non-starch polysaccharides, it is of great importance to have the most complete characteristics of structural and physicochemical properties of the samples before the experimental study. Analysis of papers devoted to exploration of the correlation between structure of non-starch polysaccharides and their pharmacological activities showed that most significant parameters are molecular weight, intrinsic viscosity, degree of esterification and galacturonic acid content. In investigation of some pharmacological effects, such parameters as total neutral sugar content and relative ratio of individual sugars may have some influence. Besides, because of the presence of rhamnose interrupting the regularity of the linear structure of the uronic acid backbone, the percentage of this sugar may play an important role in the pharmacological activities of polysaccharides (Dongowski, 1995; Hotchkiss *et al*., 2002). Because of this, in our study, we tried to make an analysis regarding all mentioned parameters of the structure of samples used in experiments. We may conclude that lead-removing effects, in such degree as it was registered, must be typical of only the substances with structure and physicochemical properties as it was defined before experiments. The results obtained through the present study suggest that pectins with low degree of esterification and calcium salt of alginic acid may be considered as perspective source for creation of novel pharmaceuticals and food agents preventing and healing lead-induced injuries in human.

CONCLUSION

In conclusion, many chelating agents are currently used to manage lead toxicity. Several and, at the same time, the most common, however, are nonspecific and have some adverse effects in humans such as induction of misbalance of essential microelements (Kalia and Flora, 2005). Because such non such polysaccharides as pectins and alginates are both specific and effective in complexing with lead, these compounds may be considered as nutritional products that could be used for decrease of lead intestinal absorption, prevention of lead accumulation and amelioration of lead toxicity. However, additional studies in rats and humans are required before developing the non-starch polysaccharides as preventive or curative agents of lead exposure and toxicity in humans.

ACKNOWLEDGMENT

This manuscript is based on the research supported by the Russian Ministry of Science and Education, Federal Grant-in-Aid Program "Human Capital for Science and Education in Innovative Russia" (Agreement No. 8064).

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