World Journal of Pharmaceutical research

Volume 2, Issue 3, 500-510. Research Article ISSN 2277 – 7105

THE AMELIORATIVE EFFECT OF *CARROT PECTIN* AGAINST LEAD ACETATE INDUCED RENAL AND HEPATIC TOXICITY IN RATS: BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY

Ouardia Ouldali[1,2], A. Aoues[2], B. Meddah[1], O. Kharoubi [2]

[1] Department of Biology, Laboratory for Research on Biological Systems and Geomatics, Faculty of Nature and Life Sciences, University of Mascara, Algeria

[2] Department of Biology, Laboratory of Experimental Biotoxicology, Biodepollution and Phytoremediation, Es-Senia University, Oran, Algeria.

Article Received on
02 February 2013,

Revised on 10 March 2013,
Accepted on 30 March 2013

***Correspondence for Author:**

Dr. Ouardia Ouldali,
Department of Biology, Laboratory for Research on Biological Systems and Geomatics, Faculty of Nature and Life Sciences, University of Mascara, Algeria.
oouldali@yahoo.fr,

ABSTRACT

Background: Poisoning resulting by the ingestion of lead containing products is an important toxicological concept in pediatric age group. ***Aims:*** In sub-acute lead poisoning, decontamination options as curative treatments are limited in efficacy. This two steps study is used to show the effects of pectin, which is a natural adsorbent on the chelation of lead and the reduction of the toxic effect in rats. ***Methods and Materials:*** The adverse effects of lead toxicity were observed on liver and kidney, more precisely, the increased activity of transaminases and the renal markers. Urea and creatinine have been demonstrated after one month of oral lead administration. ***Statistical analysis used***: To compare the different experimental groups, analysis of variance (ANOVA) was used following post *hoc* Tukey's test. The level of significance between the groups was set at $P<0.05$. ***Results and Conclusions***: The addition of carrot pectin at 3%, in the feeding of intoxicated rats, showed chelating and correcting effects on liver and kidney disturbances that were caused by lead toxicity. The findings of the present study suggest that the possible oxidative stress and histopathological abnormalities induced by lead can be neutralized by *pectin* in the lead exposed population.

Key words: pectin; lead toxicity; kidney; liver, biochemical; histopathological.

INTRODUCTION

Lead is one of the largest environmental medicine problems in terms of subjects number of exposure and public health.[1],[2] Lead caused environmental contamination including industrial producing lead and metal recycling.[3] Lead poisoning is an illness caused by increased levels of the lead in the body. The most important clinical signs in sub-acute lead poisoning are: pain, muscle weakness, araesthesia, weight loss and gastrointestinal problems. [4], [5]

The accumulation of lead in the liver causes disorders of hepatic biochemical parameters, i.e., this disorder results in cytolysis; demonstrating the alteration of the hepatocyte membrane integrity and defines the concept of hepatocyte necrosis. So, this entire disturbance was related to the destruction of hepatocytes. Many animal studies have shown that chronic administration of lead compound induced chronic interstitial nephropathy, which later progress to atrophy and fibrosis. The most characteristic cellular effect was the formation of intranuclear inclusions in proximal tubular epithelium, formed as lead-protein complex (about 50 micrograms lead/ mg protein) and would reflect a coping mechanism or protection in the transcellular transport of lead. The inclusions were scarce when atrophy and interstitial renal fibrosis worsens. [6]

Although chemical chelation was one of the therapies, it is expensive and unreliable. Nowadays medicinal plants are gaining popularity to provide comfort to various human diseases. The objective of nutrition therapy was to improve or maintain the good quality of life, nutritional status, physiological health and its prevention or the treatment of complications in the short and long-term poisoning.

Unlike other nutrients are modified during digestion, plant fibers were distinguished by the resistance to digestion and absorption in the intestine and underwent a complete or partial fermentation in the colon.

Many positive effects on health were noted such as regulation of blood glucose, serum cholesterol, and acceleration of intestinal transit, protection against cancer and other diseases of the colon through the action of chelation. [7]

In our study, we used the purified pectin molecules to remove one of the heavy metals that were tested on animals or humans.[8],[9] Moreover, in order to control the efficacy of pectin on

the heavy metal exposure, administration of pectin should be carried out alone. In this context, the aim of this work was to evaluate the effect of carrot pectin on lead toxicity in rats, following the evolution of hepatic and renal parameters (AST, ALAT, PAL, Urea, Creatinine) in addition to a histopathological study of liver and kidney.

MATERIALS AND METHODS

Chemicals and plant source

Lead acetate $(CH_3CO)_2$, a white crystalline toxic solid, and all other chemicals used in this study were of analytical grade and purchased from reliable firms like MERCK (Germany).

The vegetable was collected from supermarket of Mascara. It was identified as (*Daucus carota var muscat*) by a plant taxonomist. The pectin was extracted from carrot by the method of Naudin [10], based on mechanical (milling), physical (steam treatment under partial vacuum) and chemical (acidification with citric acid) processing. Finally, the dried product of powder form was ready for use.

Animals

Female Wistar rats (n=44), weighing 100±10g, aged 2 months were used in our study, which was performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of Mascara University, Department of Biology, Algeria. The rats were housed in a temperature-controlled room (25^oC) with 12h light/dark cycles and received a standard diet and water. All rats were fasted for 12 hours before the experiments.

Study Design

This study composed of two periods of pharmacokinetics: first period was lead exposure (1 month), followed by a second period of introduction of pectin orally (1 month).

Pharmacokinetic experiment

Rats were randomly divided into two major groups: group 1 consisted of 16 rats, designated as control, which were received distilled water and standard food for 1 month (group 1a) and 2 months (group 1b). Group 2 consisted of 26 rats that were received oral lead acetate (in water with concentration of 350 mg/l) for 1 month; six rats were sacrificed at the end of the first month (group 2a), while 10 rats were sacrificed after 2 months (group 2b) and the other 10 rats were received pectin twice a day (3%) since the 31^{th} day, and they were sacrificed at the end of the second month (group 2c).

The organs (liver and kidney) were removed, cleaned, and washed with phosphate buffer saline (pH 7.4) for various biochemical variables and histopathology/histological studies.

Analysis

Biochemical analysis

Centrifugation at 3000 rpm for 20 min was carried out to obtain serum, frozen in liquid nitrogen and then stored at -80ºC until biochemical measures. Tissue enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel. [11] Alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined by using the method of King and Armstrong. [12]

Histopathology/histological analysis

Histological evaluation of liver and Kidney was done according to the method of Mc Manus and Mowry.[13] Liver and kidney fragments were fixed in Bouines solution, dehydrated in an ethanol series, and embedded in paraffin wax for histological procedure. Liver was cut in order to obtain representative section of the organs. Paraffin sections (6 μm thick) were stained with hematoxylen and eosin. Liver and kidney histological changes were studied from the stained sections via microscopic level.

Statistical analysis

The data was expressed as mean ± SEM and analyzed using the Statistical Package for Social Science program (S.P.S.S. 11). To compare between the different experimental groups, analysis of variance (ANOVA) was used followed by ***post hoc*** Tukey's test. The level of significance between groups was set at $\boldsymbol{P}<0.05$.

RESULTS

The animals in all groups did not show any abnormal behavior except that lead-exposed animals were less active and more irritable. Moreover, no significant differences were registered in the body weights of the animals from different group treatments, and none of them died during experimental period.

Biochemical assays

Lead exposure for 30 days produced disturbance of the serum liver enzyme activities as compared to the control group, with a significant increase (P<0.05) in AST, ALT and ALP At the end of the second period (30 days), after the adding of carrot pectin (group 2c), there was a regularizing effect of serum liver enzymes compared to control groups (group 2b) and (group 1b): 15.05% decrease (p <0.005) in AST, decrease of 26.4% (p <0.005) for ALT and an decrease of 7.27% for PAL (p <0.001).

Serum markers urea, creatinine were used to evaluate renal disease in the subject exposed to lead**.** [14], [15] Uremia, at the end of the period of lead exposure, was different in the group treated with lead compared to the control group as clearly seen by an increase of urea to 13.33% (P>0.005) and for creatinine an increase of 38.24 % (p<0.005). According to Michaux [16]**,** this would be the consequence of a reduction in the glomerular filtration rate.

At the end of the second period (30 days), after the adding of carrot pectin, there was a corrective effect (decrease of 113when comparing the group treated with the pectins to the other groups not treated. The values of Uremia and creatinine are showed in the table n°01. Well, according to El Zoghbi and Sitohy, uraemia and creatinine of rats poisoned with lead, returns to a normal value after treatment with low-esterification pectins. [7]

Table No. 01: Results of biochemical's essays

Period of essay	1st period		2nd period		
Groups Parameters	1_a	2_a	1_b	2_b	2_c
AST (UI/l)	70.83±1.72	100.54±0.50	80.03±1.95	107±4.96	90.89±7.36
ALT (UI/l)	31.4±1.04	42.81±1.16	35.37±1.66	48.06±1.76	37.09±6.04
ALP (UI/l)	177.02±2.05	194.69±2.05	191.94±2.94	210.97±3.62	195.63±2.58
Uremia (mmol/l)	0.45±0.01	0.51±0.08	0.50±0.02	0.60±0.02	0.53±0.1
Creatinine (mmol/l)	8.68±0.24	12 ± 0.11	10.15±0.03	15.51 ±0.99	13.42± 0.44

Histopathological/histological studies

Histological evaluations of liver and kidney tissue in different groups were done in the sections stained with hematoxylen and eosin.

➢ **Liver tissue**

Group *1$_b$* *(control, untreated, normal animals):* Liver of these animals displayed normal architecture, where typical aspects, i.e., lobular pattern with prominent central vein and patient sinusoids were evident (Figure1)

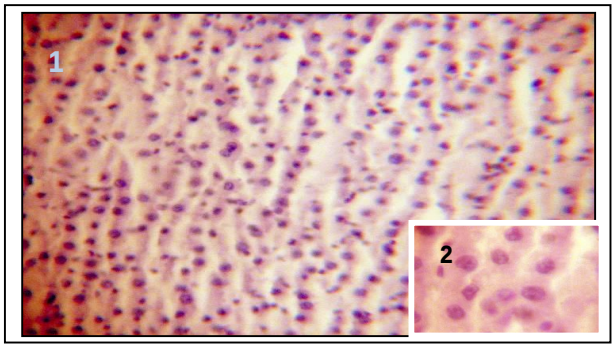

Figure 1: T.S. liver of control group 1_b
1: (H and E x170)
2: (H and E x680)

Group *2$_b$* *(lead-treated animals):* Lead exposure produced clear hepatic histopathological alterations in liver including focal necrosis with inflammatory cells, congestion at places, increased number of sinusoids, centrilobular swelling, hepatocyte vacuolation and swelling, parenchyma disorganization, dilation of the inter hepatocyte space, and hemorrhagic clots when compared to group 1_b (Figure 2).

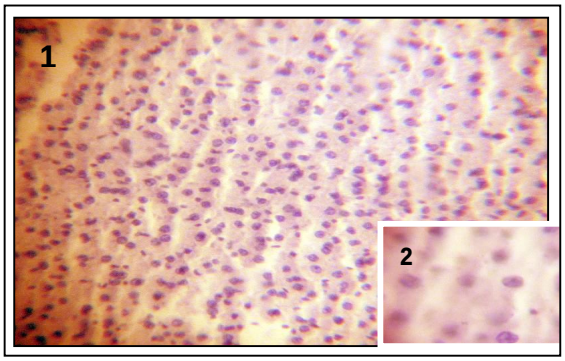

Figure 2: T.S. liver of lead-treated group 2_b
1: (H and E x170)
2: (H and E x680)

Group *2c* *(pectin treated animals):* Animals of these groups showed no histological differences comparing with group 1_b, except low vacuolation and disorganization. Then pectin showed remedial effect in this organ against lead toxicity (Figure 3)

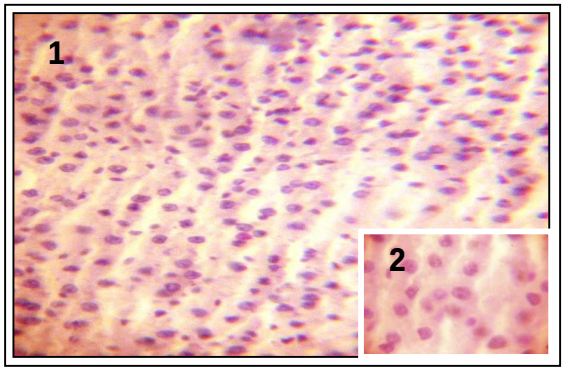

Figure 3: T.S. liver of lead-treated + pectin group 2c
1: (H and E x170)
2: (H and E x680)

Histological examination of livers of rats (group 2c) reveals a normal histological appearance almost identical to that of control rats (Group 1_b). It indicates no plaques that observed in positive control rats (group 2b) with an increased number of hepatocytes and hepatocyte trabeculae.

Results were confirmed by Serguschenko[17] indicated that pectins have the ability to bind metals such as lead; the researchers suggested that pectins influence metabolism and lead poisoning.

- *In vitro*, low esterified pectins set 2 mmol of lead per gram of pectin (relative to calcium pectates and highly esterified pectins)
- Treatment of rats by low-esterified pectins or calcium pectates, reduced retention of lead by the heart, liver, kidneys and bones.
- Pectins reduced the lead concentration in the tissues of organs.

Kidney tissue

Group *1* *(control, untreated, normal animals):* In the kidneys of control group 1, the histological examination showed a normal appearance of glomeruli and tubules. (Figure 4)

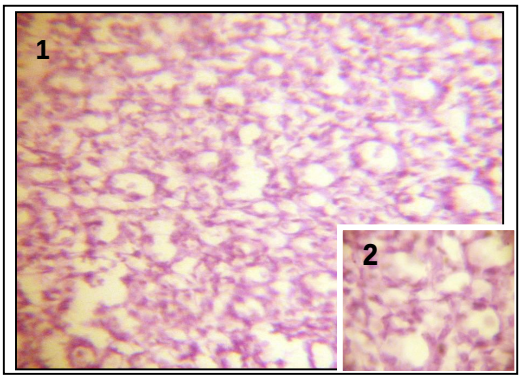

Figure 4: T.S. kidney of control group 1
1: (H and E x170)
2: (H and E x680)

Group *2_b* *(lead-treated animals):* However, morphological changes on the Kidney of lead-treated group 2b showed an increase in the surface of the tubules (Figure 5).

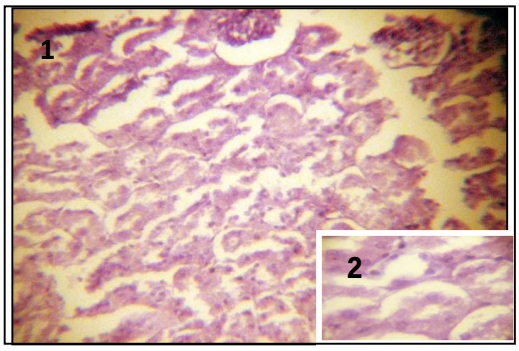

Figure 5: T.S. Kidney of lead-treated group 2_b
1: (H and E x170)
2: (H and E x680)

Group *2c (pectin treated animals):*

Histological study of the kidney of rats treated with pectin (group 2c) indicated a significant decrease in the surface of the tubules compared to the kidneys of rats treated with lead (group 2b). The decrease in the surface of the tubules is assumed to be a phenomenon noticed in the case of the detoxifying effect of carrot pectins against lead (figure 6).

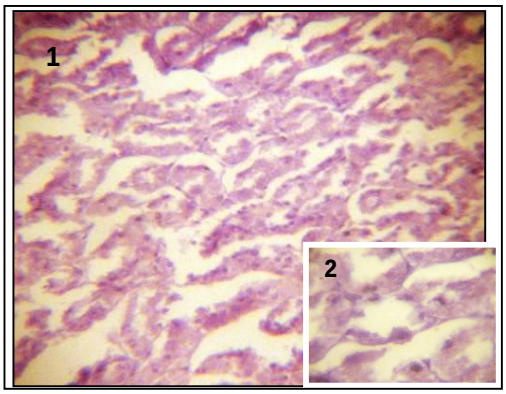

Figure 6: T.S. Kidney of lead-treated + pectin group 2c

1: (H and E x170)
2: (H and E x680)

Our results corresponded to the work of several authors Albahary [18]; Lillis[19]; Goyer [20], [21]; Khalil-Manesh [22], [23], [24] and Loghman-Adham [25], who showed that the renal toxicity of lead occurs mainly by morphological and functional damage of proximal tubules with reduced capacity for absorption of low molecular weight compounds.

Thus, Albahary [18], Bennett [26] and Weaver [27] showed the presence of alterations of tubular kidney biopsies of subjects with lead exposure which are high and consist with the results. According to Loghman-Adham [25], acute renal manifestations are usually reversible after cessation of lead exposure and chelation therapy.

CONCLUSION

To conclude, our results are confirmed by other studies *in vitro*, showing that the pectin can form complexes with cations, di- and trivalent.[18] A similar effectiveness has also been observed among children living in an environment contaminated by a large number of toxic chemicals (lead, arsenic, copper, chromium and cadmium). [28]

The results of the positive effect of pectin on the chelating of lead were reported [28], [29] confirming that treatment with pectin reduced the toxic action of lead in blood, significantly ($P<0.01$), and that, finally, the pectin has a corrective role against lead toxicity.

Based on diverse research that was already cited, the effect of pectin from different vegetables showed an interesting result in the field of lead phytochelation[28], which could

have a therapeutic effect particularly among the most sensitive young subjects as preventive and/or curative treatment of heavy metal poisoning, especially for lead, by trapping of a partial or all the metal and facilitate its elimination via the feces.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

Many thanks to Mr. Medarbal KH for his inputs to this study.

REFERENCES

1. Ragan P, Turner T. Working to prevent lead poisoning in children: Getting the lead out. JAAPA 2009; 22: 40-5.
2. Pokras MA, Kneeland MR. Lead poisoning: Using transdisciplinary approaches to solve an ancient problem. EcoHealth 2008, 379-85.
3. Mañay N, Cousillas AZ, Alvarez C, Heller T. Lead contamination in Uruguay: the "La Teja" neighborhood case. Rev Environ ContamToxicol 2008, 195: 93-115.
4. Rossi E. Low level environmental lead exposure -a continuing challenge. Clin Biochem Rev 2008, 29: 63-70.
5. Meyer P, Brown M, Falk H. Global approach to reducing lead exposure and poisoning. Mutat Res 2008, 659:166-75.
6. Beliles R.P**.** The Metals. In: Clayton G.D., Clayton F.E. (eds.) Patly's Industrial hygiene and toxicology II; Part C. New York 1991,7; 206.
7. El-Zoghbi M, Sitohy MZ. Mineral absorption by albino rats as affected by some types of dietary pectins with different degrees of esterification. Nahrung 2001; Pp 114-7.
8. Le Gall B, Tarrana F, Renault D, Wilk JC, Ansoborlo E. Comparison of Prussian blue and apple-pectin efficacy on 137Cs decorporation in rats. Biochimie 2006; p 1837-41.
9. Degtiareva TD, Katsnel'son BA, Pirvalova LI, Beresnova Olu, Gurvich VB, Kuz'min SV. Use of biologically active substances in preventing the toxic action of some heavy metals. Gig Sanit 2001 p 71-3.
10. Naudin O, Josselin P, Baynast RD. Process for the production of food fibers from carrots and fibres contained by this process1992; Application Number: EP19910402240.
11. Reitman S, Frankel AS**.** A colorimetric method for the determination of serum Glutamic oxaloacetic and Glutamic pyruvic transaminase. Am J Clin Path 1957; p 53–6.

12. King E J, Armstrong. 2-4 DNPH method of determination of Serum GOT and GPT. Can Med Assoc J 1934; p31- 326.
13. Mc Manus FA, Mowry RW. Staining methods. In: Hoeber PB, editor. Histology and Histochemistry1965. New York: Harper and Brothers;
14. Weaver V.M., Lee B.K., Ahn K.D., Lee G.S., Todd A.C., Stewart W.F., et al. Association of lead biomarkers with renal function in korean lead workers. Occup. Environ. Med 2003[a]; p 60 - 551.
15. Weaver V.M., Jaar B.G., Schwartz B.S., Todd A.C., Ahn K.D., Lee S.S.,Wen J., *et al*. Associations among lead dose biomarkers, uric acid, and renal function in Korean lead workers. Environ-Health Percept 2005. p 113 - 36.
16. Michaux P., Boiteau H.L., Tolot. Valeur et limite de dépistage clinique et biologique en pathologie industrielle. Arch. Mal. Prof 1971**.** p 1-2 ,56-66
17. Serguschenko I., Kolenchenko E., Khotimchenko' M. Low esterified pectin accelerates removal of lead ions in rats. Nutrition Research 2007 ; Volume 27; Pp 633–639
18. Albahary C., Richet G., Guillaume J., Morel-Morger L. le rein et le saturnisme professionnel. Arch. Mal. Prof 1965. Pp 26 -5.
19. Lilis R., Gavrilescu N., Nestorescu B., Dumitriu C., Rovanta A. Nephropathy in chronic lead poisoning. Br. J. Ind. Med. 1968. Pp 25 - 196.
20. Goyer R.A. Mechanism of lead and Cadmium nephrotoxicity. Toxicol. Lett 1989; Pp 46-153.
21. Goyer R.A., May P., Cates M.M., Krigman M.R. Lead and proteins content of isolated intranuclear inclusion bodies from kidney of lead poisoned rats. Lab. Invest 1970[a]; Pp 22- 245.
22. Khalil –Manesh F., Gonick H.C., Cohen A.H., Alinovi R., Bergamashi E., Mutti A. Experimental model of lead nephropathy I: continues high-dose lead administration. Kidney. Int 1992[a]; Pp 41 - 1192.
23. Khalil –Manesh F., Gonick H.C., Cohen A., H. Alinovi R., Bergamashi E., Mutti A. Experimental model of lead nephropathy. II. Effect of removal from lead exposure and chelation treatment with dimercaptosuccinie acid (DMSA). Environ.Res 1992[b]; Pp58-35.
24. Khalil –Manesh F., Gonick H.C., Cohen A.H. Experimental model of lead nephropathy. III continues low-dose lead administration. Kidney. Int 1993[a]. pp 48- 271
25. Loghman-Adham M. Renal effects of environmental and occupational lead exposure. Environ. Prespect 1998; Pp 105-928.

26. Bennett WM. Lead nephropathy: Nephrology forum. Kidney Int 1985. Pp 212-20.
27. Wedden R. Renal diseases of occupational origin. Occup. Med 1992. Pp : 449-463
28. Virginia M. Weaver,[1,2] Bernard G. Jaar,[2,3] Brian S. Schwartz,[1,2,3] Andrew C. Todd, *and al* . Associations among Lead Dose Biomarkers, Uric Acid, and Renal Function in Korean Lead Workers. Environ Health Perspect 2005; pp36–42.
29. Trakhtenberg IM, Lukovenko VP, Korolenko TK, Ostroukhov VA, Demchenko PI, Rabotyaga TY, *et al*.. Preventive use of pectin for chronic exposure to lead in production. Lik Sprava 1995. Pp 132-5.
30. Tahiri M, Doco T, Tressol JC, Pellerin P, Pepin D, Rayssiguier Y, *et al*. The Rhamnogalacturonan II, a pectin polysaccharide, decreases intestinal absorption and tissue retention of lead in rats. Bulletin de l'Office International de la Vigne et du vin 2001. Pp 228-35.