ORIGINAL ARTICLE

Beneficial effects of carrot pectin against lead intoxication in Wistar rats

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The aim of the present study was to investigate the beneficial action, *in vivo*, of pectin against subacute lead acetate (350 mg/l) intoxication. The adverse effects of lead on the haematological disturbances that concerned, more precisely, the decrease of red blood corpuscle life duration and on the appearance of ever granulated basophilic haematites by inhibiting an enzyme responsible for haeme synthesis have been demonstrated after 1 month of oral lead administration to female Wistar rats. Also, this caused an elevation of the blood lead level as compared with the control group. The introduction of carrot pectin to a level of 3% in the feeding of intoxicated rats has shown a chelating and correcting effect on haematological disturbances caused by lead toxicity, which is reflected by a significant decrease ($P<0.05$) of blood lead (from 117 to 65 to 19 µg/l), zinc protoporphyrine (portophyrine-zinc from 7.7 to 5.1 to 3.5 µg/g of Hb), increase in haemoglobin to 27% (from 5.09 to 6.05 to 7.79%) and iron to 8% (from 1.34 to 0.9 to 0.5%) of the treated rats by pectin as compared with the untreated groups. Differences in blood lead were significant between the control diet and the addition of pectin therefore suggesting that pectin fibre ingestion in diets decreases the risk of lead poisoning.

Key words: Blood lead, carrot pectin, haemoglobin, lead acetate, ZPP

INTRODUCTION

Lead is one of the largest environmental medicine problems in terms of numbers of exposure of subjects and the public health.[1,2] Lead-caused environmental contamination included industrial producing lead and metal recycling.[3] Lead poisoning, known as plumbism, colica pictonium, is a medical condition caused by increased levels of the heavy metal lead in the body. The most important clinical signs in subacute lead poisoning are pain, muscle weakness, paraesthesia, weight loss and gastrointestinal problems.[4,5] Lead was responsible for many physiological disorders as well as the protein biosynthesis disturbing inhibitory action on haemoglobin synthesis, causing a kind of anaemia and the increased level of acid-δ-aminolevulinic, which was a neuropathogenic agent.[6,7] However, the substitution of zinc with lead in four active sites of cysteine residues, the δ-aminolevulinic acid deshydratase (ALAD), was altered so that its biological activity prevented the formation of porphobilinogen, an intermediate key of biosynthesis central to the fixation of oxygen in haemoglobin. The replacement of Zn^{+2} ions by Pb^{+2} sites linked to the metalloenzyme blocked the functioning of ALAD, the δ-aminolevulinic acid synthetase (ALAS) and ferrochelatase.[8] The amount of blood lead was considered as a good indicator of recent exposure, and determined organ toxicity.[9] Therefore, the portophyrine-zinc (ZPP) determination was not applied only to high exposures but was also as an index of the effectiveness of chelating therapy,[10] and the formed chelate must be non-toxic such that it can be removed from the body by excreting in urine.[11,12]

The objective of nutrition therapy was to improve or maintain the quality of life, nutritional status, physiological health and the prevention or the treatment of complications in the short and long-term poisoning. Unlike other nutrients that are modified during digestion, plant fibres were distinguished by the fact that they were resistant to digestion and absorption in the intestine and underwent a complete or partial fermentation in the colon.

Many positive effects on health are noted, such as regulation of blood glucose, serum cholesterol, acceleration of intestinal transit, protection against cancer and other diseases of the colon through the action of chelating.[13]

In our study, we used the purified pectin molecules to

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remove one of the heavy metals that was tested on animals or humans.[14,15] Moreover, in order to rule out the efficacy of pectin on the heavy metal exposure studies, it must be conducted in carrying out the administration of pectin alone [Figure 1]. In this context, the aim of this work was to evaluate the effect of carrot pectin on lead poisoning in Wistar rats, following the evolution of haematological parameters (PbB, ZPP, Hb and iron serum).

MATERIALS AND METHODS

Forty-four Wistar rats weighting 100±10g (98–122 g), aged 2 months and of female sex were selected for this study. The experiment was approved by the ethical committee. The substance that was used in the experiment was carrot pectin, which was extracted by the method[16] 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. Rats were isolated and treated individually in appropriate cages. They were exposed to a daily photoperiod of 12 h and temperature of 23±2°C. They were fed on a standard diet and water was renewed daily. After the period of habituation, rats were randomly divided into two major groups: group I consisted of 16 rats, designed as control, which received distilled water and standard food for 1 month (group Ia) and 2 months (group Ib). Group II consisted of 26 rats that 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 IIa), while 10 rats were sacrificed after 2 months (group IIb) and the other 10 rats received, since the 31th day, pectin twice a day (3%), and they were sacrificed at the end of the second month (Group IIc).

Total blood and serum were frozen in nitrogen liquid and then stored at -80ºC until biochemical measures. The blood lead level, ZPP activity and blood smears were determined. To determine the serum iron, we have used centrifugation at 3000 rpm for 20 min to obtain serum.

Determination of Blood Lead Levels

Blood lead is the best indicator of stable lead exposure for the previous weeks. The result of a limited measure reflects recent exposure blood lead levels that decreased progressively, with a half-life for approximately 30 days.[17]

The analysis is conducted at a laboratory approved for the analysis of heavy metals in biological matrices Toxilabo of Nantes in France.

The method of analysis used for blood lead was by using a spectrophotometer ((PerkinElmer, Inc., Shelton, CT)/ NV2071 model 41102L. AA) and a graphite furnace Zeeman that worked at a wavelength of 283.3 nm. According to the method of standard additions, the limit of sensitivity was 50 µg/l. The result is expressed as mg/l.

Determination of Zinc-Portophyrine

The use of ZPP is possible because it correlated very well with blood lead and represented the earliest indicator of lead poisoning. On the other hand, it did not lend itself to contamination outside the inverse.[18] To determine ZPP, we have used haematofluorimetry AVIV- ZPP flouorometer (model 206), LAKEWOOD, NEW JERSY (USA) (model 206) in the laboratory Toxilabo of Nantes; the wavelength of excitation was 450 nm and the measurement of fluorescence was 600 nm. The reading is expressed in micro mol (ZPP/mol haeme or g ZPP/g haemoglobin).

Haemoglobin (Sahli method)

Lead causes a disturbance on the anabolism of haeme by a severe inhibition of the activity of ALAD.[19] All forms of haemoglobin are converted into haematin hydrochloride by the action of hydrochloric acid, and the intensity of staining is directly proportional to the concentration of haemoglobin identified by specific markings of the aircraft-Sahli haemoglobin meter. The result is expressed as a percentage (%).

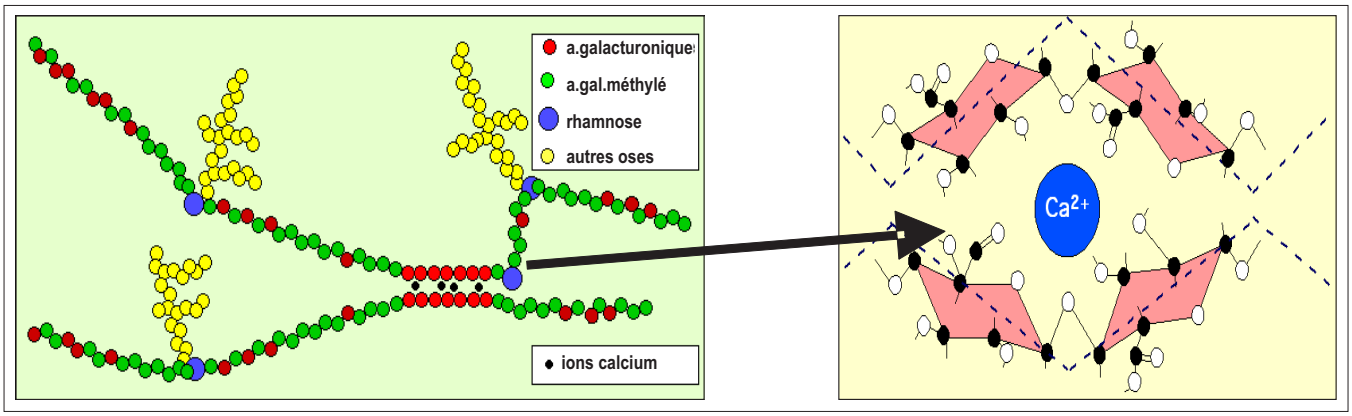

Figure 1: Binding two pectin chains = Training the "egg box"

Determination of Serum Iron (Spinreact)

Anaemia Sédéroblastique is secondarily acquired as hypochromic and microcytic. It is the result of two mechanisms: haemolysis and a slight decrease in the synthesis of haeme. Indeed, the presence of lead inhibits several enzymes in the biosynthesis of haeme; the amount of iron incorporated in haeme decreases, which explains the rate of Fe^{+2} serum level.[20]

The principle of the method is based on the reaction of iron with the working solution, and iron ferina gives the iron concentration in the blood. The absorbance of the sample is read beside the blank at a wavelength of 562 nm, and the result is expressed as follows:

$$\text{Fer (g/dl)} = \frac{\text{Echantillon - blan}}{\text{Standard}} \times 100$$

The Blood Smear (May-Grünwald Giemsa)

The blood smear is recognized to examine the single-cell spread in blood elements. The reading microscope with set goals and 100× by using a calibrated objective was used to measure the diameter of erythrocytes.

The morphological action of lead was manifested by early presence in the bone marrow and blood consequently in reticulocytes, which are atypical to the erythrocyte basophilic granulation (HGB).

The basophilic granules in erythrocytes due to lead poisoning gave a red cell abnormal residue youth-specific reticulocyte cell with an extra character that was not physiological, the agglomeration in clump, and not within its complex of polysomes, effecting the basic colour. These pitted red cells found in the bone marrow in the early days of lead impregnation before the disappearance of defective blood, captured by the reticule endothelial system, explain that they are found very normally in subjects intoxicated and out of the toxic action.[1] In the erythrocyte, lead inhibited pyrimidine-5-nucleotidase, the enzyme for protein synthesis found in the cytoplasm of the erythrocyte,[20] and this inhibition allowed the haemolysis of red blood cells.[19]

Statistical Analysis

Analysis of variance (ANOVA) and –Student's t test were performed to compare the prophylactic effect between groups and within the same group. In all the tests, the *P*-values of less than 0.05 have been considered significant. Data are expressed as means±SEM.

RESULTS AND DISCUSSIONS

It is know that once the lead is absorbed in the intestine, it passes into the bloodstream, where more than 90% is set to the level of erythrocyte membranes and haemoglobin, the rest diffusing into the serum, it is then distributed to various organs and tissues [Figure 2].

The results of analysis carried out in certain indicators of lead poisoning in blood are taken from two essential steps of sacrifices made after 30 days (confirmation of impregnation lead poisoning) and after the second period of 30 days of supplementation with pectin (audit chelating effect of using the same indicators, blood).

Blood Lead and Zinc-Protoporphyrin

With the exception of other indicators, measurements of blood lead and ZPP are performed on samples at the end of the second period after introduction of pectin. The results showed a clear indication that pectin introduced in the ration of Lot rats (IIc) had a positive effect on various indicators of lead poisoning (blood lead and ZPP) compared with the batch intoxicated (IIb), where the harmful effect of lead on haeme synthesis was very pronounced in inhibiting the three essential enzymes responsible for this mechanism that could cause anaemia [Figures 2 and 3].

Our results are confirmed by other studies *in vitro*,[13]

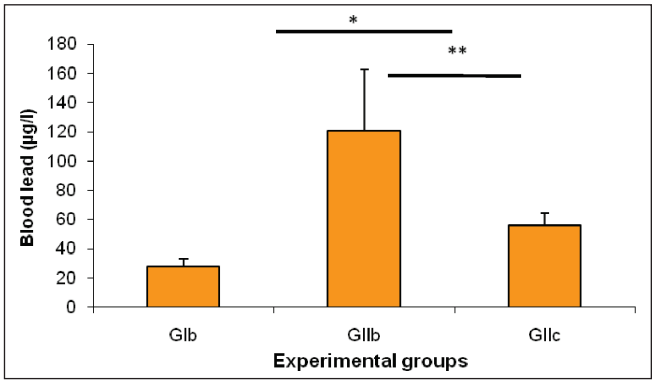

Figure 2: Changes in the levels of lead acetate (PbAc) in the blood of control (GIb), lead-treated (GIIb) and lead-treated Wistar rats given pectin fibers (GIIc). Data are mean±S.E.M. * *P*<0.05, ** *P*<0.01.

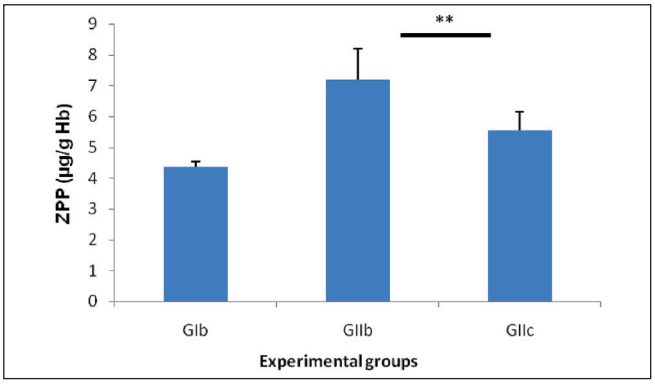

Figure 3: Changes in the levels of ZPP in the control group (GIb), lead-treated group (GIIb) and lead-treated Wistar rats given pectin fibers group(GIIc). Data are mean±S.E.M. ** *P*<0.01

showing that the pectin can form complexes with cations, di- and trivalent. A similar effectiveness was also observed among children living in an environment contaminated by a large number of toxic chemicals (lead, arsenic, copper, chromium and cadmium).[21] In addition, the study by Degtiareva *et al.* on the effectiveness of apple pectin on the cesium in rats has shown that pectin can reduce up to 53% of Cs.

Haemoglobin

The results obtained [Table 1] in the first period showed that haemoglobin decreased (-22%) in the lot (IIa) compared with that lot (Ia), resulting from the action of lead on haeme biosynthesis, which undergoes significant inhibition during lead poisoning. Lead causes a disturbance of the anabolism of haeme by a severe inhibition of the activity of ALAD, and it is considered that lead exerts an inhibition level of sulfhydryl groups of the enzyme ALAS.[1,22]

The inhibition of synthesis of globin genes influences the encoding of this synthesis and inhibition of the incorporation of glycine into globin in erythrocytes. These changes are responsible for the appearance of a saturnine anaemia.[4]

Then, during the second period (2nd month), the haemoglobin lot (IIc) is restored normal (3%) compared with the batch (Ib) and that following treatment with pectin compared with the lot (IIa), which is decreased due to the persistence of lead toxicity; i.e., that the increase in haemoglobin in the lot (IIc) is proportional to the administration or the treatment with pectin [Table 1].

Our results are similar to those of a previous work,[23] confirming that the haemoglobin found in red blood cells mature peripheral blood. This index showed the positive role of pectin in the regeneration and synthesis of red blood cells. According to statistical analysis, the result is highly significant (P<0.01).

Serum Iron

In the results shown in Table 1, we distinguish that the rate of serum iron in lot (IIa) is high (3%) compared with controls (Ia), which may explain why lead would act as competitive antagonist of iron on the ferrochelatase enzyme and cause a change in the conformation of its active site, i.e. lead alters iron metabolism by decreasing the binding capacity.

We showed that the rate of serum iron batch (IIa) witnesses were intoxicated, which remains high in the second period (2 months) compared with controls (Ib) despite having stopped treatment lead; this could explain the fact that for the remaining period of the toxicity for long term, lead causes increased serum iron, an observation also Confirmed by Tahiri M. *et al.*, 2001 Anaemia Sédéroblastique is secondarily acquired, hypochromic and microcytic, and it is the result of two mechanisms: haemolysis and the slight decrease in the synthesis of haeme. Indeed, the presence of lead inhibits several enzymes in the biosynthesis of haeme; the amount of iron incorporated into haeme decreases, which explains the rate of Fe^{+2} serum level. As it is stressed that the level of serum iron of the lot (IIc) is almost the same as the normal rate (8%) recorded in the control group. Therefore, the results of the positive effect of pectin on the chelation of lead were reported,[21,24] confirming that treatment with pectin reduced, significantly (P<0.01), the toxic action of lead in blood.

Blood Smear

There are red cells with basophilic granulation considered as an important marker to confirm the effect of lead toxicity.[6] The HGB has a life span shorter than normal reticulocytes, and the presence of erythrocytes in lots treated with lead (Pb) 4 µ in diameter is lower than those of the healthy control group (Ia) (7.2 µm). This may explain that lead causes anaemia, which results in microcytosis.

Thus, the same figure shows that the red cells of different shapes, haemolytic, i.e. there is the result of an outbreak of haemolytic anaemia due to the fact that lead causes slight haemolysis of red blood cells, with a significant presence of erythrocytes that are less colourful due mainly to the absence of haemoglobin, which reconfirms the inhibitory

Table 1: Variation in rates of haemoglobin, serum iron levels and red cell numbers in all groups

Blood parameters Groups	Taux du Fe^{+2} µg/dl	Taux de Hb%	Nombre des GR/ mm^3 ($x10^4$)
G Ia	127.75±11.87	7.79±0	411.0±4.933
G IIa	168.5±9.03	6.05±0.86	59.75±1.773
G Ib	139.28±10.82	7.00±0.87	431.03±65.565
G IIb	171.57±12.42	5.09±0.95	57.367±8.779
G IIc	15085±3.43	7.26±1.17	517.445±111.445

NS – Non-significant; SE – Standard error; *P<0.05 **P<0.01

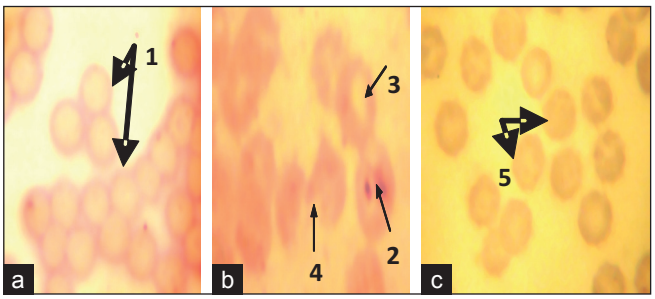

Figure 4: Photomicrograph of blood frottis of : (a) control group (G.Ib), (b) intoxicated group by lead acetate (G.IIb) and (c) treated group receiving lead acetate (350 mg/kgbwt) plus pectin fibers (3%) (G.IIc) (1700×)
1-5: normal red cells, 2: Red cells with basophilic granulation, 3: Hypochrome red cells, 4: Hemolyzed red cells

effect of lead on the synthesis of haemoglobin above the level of specific enzymes, the synthesis of haeme (ALAD, ALAS, ferrochelatase, ... etc.).

We noted that the intoxicated rats treated with pectins (IIc) have normochromic RBCs and normal size (7.2–7.6 μm) [Figure 4]. This can be explained by the fact that blood cells formed in the period treatment are normal and, therefore pectins have a corrective role of lead toxicity, an effect confirmed by previous study;[13] pectin can form complexes with cations, di- and trivalent.

CONCLUSION

Our results are confirmed by other studies *in vitro*, showing that the pectin can form complexes with cations, di- and trivalent.[13] A similar effectiveness was also observed among children living in an environment contaminated by a large number of toxic chemicals (lead, arsenic, copper, chromium and cadmium).[21] Also, confirming that the haemoglobin found in red blood cells mature peripheral blood. This index showed the positive role of pectin in the regeneration and synthesis of red blood cells.

Therefore, the results of the positive effect of pectin on the chelation of lead were reported,[21,24] confirming that treatment with pectin reduced, significantly (P<0.01), the toxic action of lead in blood and that, therefore, the pectin has a corrective role of lead toxicity.

Based on diverse research that was already cited, the effect of pectin from different plants showed an interesting result in the field of lead phytochelation, which could have a curative 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 partially or all the metal and facilitate its elimination via the faeces.

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