

Effect of Pectin Extract of Date (*Phoenix Dactylifera* L) on Erythrocytes Oxidative Damage and Hematological Parameters Induced by Lead in Males Rats

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ABSTRACT

Lead is a non-essential element for human body and can caused a several damage in all metabolic and structural function, especially on hematological system. The aim of this study was to evaluate the effects of pectin extract from date (*Phoenix Dactylifera* L) on hematological and biochemical alteration in red blood cells (RBC) induced by lead, and determination of lead burden in blood. Rats were exposed to lead acetate (350mg/Kg) for 4-weeks and treated during 4-weeks with pectin extract at 3%. Orally administration of pectin after intoxication with lead acetate increased significantly the red blood cells count (RBC), hemoglobin (Hb), hematocrit (Ht), means corpuscular hemoglobin (MCH) and decreased significantly white blood cells count (WBC) in intoxicated group vs. control. Moreover, pectin extract of date improved δ -aminolevulinic acid dehydratase (δ -ALAD) activities in rats treated compared with the intoxicated rats, and reduced blood lead level. Therefore, pectin provided a significant protection to Thiobarbituric acid reactive substances (TBARS) level in erythrocyte whereas caused an increase in superoxide dismutase (SOD) and glutathione reductase (GSSH-Red) activities with a decrease of glutathione peroxidase (GSH-Px), glutathione transferase (GST) and Glutathion reduced (GSH) level in red blood cells. In conclusion, the data suggests that pectin may have the ability to chelating lead and reducing oxidative stress in erythrocyte.

KEYWORDS: Lead acetate, Pectin, *Phoenix Dactylifera* L, Hematology, Erythrocyte, Oxidative stress.

INTRODUCTION

Lead is a persistent and common environmental contaminant that can be found in food and water; hence, its toxicity remains an important public health issue. Several adverse effects of lead acetate are widely documented on different organs such as the reproductive system¹, nervous systems [2], kidneys [3], liver [4] and genotoxicity [5]. The signs of disorders are similar to those of antioxidant deficiency [6]. Therefore, the treatment against lead toxicity with administration of non-enzymatique antioxidant is usually used [4]. Nowadays, Phytotherapy remain a very interesting domain to explore cause it's has been proved that medicinal herbs are effective, safe and lesser in cost [7]. However, the more reasonable method with the purpose of elimination of metals is the use of chelate therapy [6]. In fact, pectin has a great affinity for binding to metal ions [8]. The fruit of palm date (*Phoenix dactylifera* L), considered as an ideal food rich in nutrients [9], carbohydrates, dietary fibers like pectin substances, certain essential vitamins and minerals [10].

Algeria is considered one of the date producing countries. The quantities of date produced annually in Algeria is around 500 000 tones [11], the essential part of this product is a dry dates, which are characterized by low marketable value, while the overproduction of date causes a problem of marketing for the cultivators. Generally, dry dates usually transferred for cattle feeding [12].

The aim of this study was to investigate the effects of pectin extract of date «*Phoenix Dactylifera* L» on oxidative stress induced by lead exposure on hematological system and biochemical parameters in male albino rats.

MATERIALS AND METHODS

Plant Material

Fruits of *P. dactylifera* (Arecaceae) variety Deglat beida were collected in 2013 in palm grove in Southern of Algeria (Bechar) at the « Tamr stage » which were characterized by dry dates allowing easy preservation. The fruits were identified taxonomically and authenticated by the Botanical Research Institute Mascara University (voucher specimen number: *P. dactylifera* L-448). After petting, the date fleshs were dried and ground to obtain a powder of date.

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Extraction of Pectin

The extraction was done following the method of Nilesh [13], with a little modification. Briefly, 100 grams of the dried date powder was suspended in 600ml of distilled water. Then, the mixture was acidified by using nitric acid (53%) to obtain a pH of 1.5. Extraction was done at 95° C for 60min. After cooling, the mixture was centrifuged twice and the supernatant was separated from insoluble residue. The pectin solutions previously adjusted to pH of 3.5 with 10 M NaOH were precipitated with an equal volume of ethanol (96%). After filtration, the insoluble pectin was washed twice with ethanol (70%) to remove impurity. The precipitate pectin was then evaporated by using Rotavapor to eliminate the ethanol and freeze-dried.

Analytical methods of pectin

Galacturonic acid (Gala) content

Total galacturonic acid was determined colorimetrically by using a modified m-hydroxydiphenyl sulfuric acid method [14]. The colored forming product was measured by spectrophotometer using D galacturonic acid as a standard.

Determination of Degrees of esterification (DE)

The content of methanol in pectin was investigated by using enzymatic treatment with alcohol oxidase at 412 nm [15]. The degree of esterification was obtained from the molar ratio of methanol to galacturonic acid.

Animals

Fifty male Wistar rats weighing 100 ± 5 g were housed in Experimental Bio-Toxicology, Bio-Depollution and Phyto-Remediation Laboratory, Department of Biology, University of Oran 1 (Algeria) under standard environmental conditions ($23 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity and a 12 h light/dark cycle) and maintained with free access to water and a standard diet *ad libitum*.

Experimental design and sacrifice

Fifty rats were randomly divided into 2 groups of rats: control group C1 (20 rats) and intoxicated group Pb1 (30 rats) that received 350 mg/kg of lead by gavage. After one month of the experiment and overnight fasting, 10 rats of each group (group Control « C1 » = 10 rats, group given lead acetate solution « Pb1 » = 10 rats) were sacrificed after be anesthetized with chloral solution (3 ml/kg body weight). The rest of rats from C2 group receive distilled water, and the half of intoxicated rats received distilled water (Pb2 group). The second half of intoxicated rats received pectin solution orally at dose of 3% (Pb+P group).

After sacrifice, blood was collected from the abdominal aorta into dry tubes for estimation of lead levels, for hematological study, the blood samples were drawn using EDTA as an anticoagulant. Serum and plasma were prepared by centrifugation (3000g for 20 min, 4°C). For determination of ALAD activity, the red blood cells were washed three times with an equal volume of cold physiological saline (0.9% NaCl). For oxidative stress study, erythrocytes were washed three times with an equal volume of PBS (phosphate buffer solution). The samples were maintained at -80°C before performing assays.

Lead estimation

The concentration of lead in blood was esteemed by using Spectroscopic Atomic Absorption method (Shimadzu AA-6200 flame) in AFAK Control Center (Oran, Algeria).

Hematological assay

Red blood cells count ($\text{RBC} \times 10^6 \text{ mm}^{-3}$), white blood cells count ($\text{WBC} \times 10^3 \text{ mm}^{-3}$), hemoglobin concentration (Hbg/dL), hematocrit (Ht%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were quantified in an automatic hematological assay analyzer.

ALAD activity

Erythrocyte δ -ALAD was esteemed by the method of Mauzerall [16]. Erythrocyte δ -ALAD acts on aminolevulinic acid (ALA) to form porphobilinogen (PBG), which is further reacted with modified Ehrlich's reagent to form a pink-colored compound, which was measured at 555 nm.

Measurement of lipid peroxidation

Oxidative damage in erythrocytes was assessed by measuring the rate of lipid Thiobarbituric acid reactive substances (TBARS) which were determined by a method based on the reaction of Thiobarbituric acid (TBA) with Malondialdehyde (MDA) or MDA-like substances to produce a pink pigment with an absorbance maximum at 532 nm [17,18].

Measurement of antioxidant enzyme activities and molecules

Reduced glutathione (GSH) content in red blood cells was determined following the method described by Sedlak [19]. Which is based on reaction of oxidation of GSH by 5, 5' Dithiobis 2-nitrobenzoic acid (DTNB), therefore liberating of thionitrobenzoic acid (TNB). Superoxide dismutase (SOD) activity in erythrocytes was assayed by the use of tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (kit, Cayman, USA). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical and expressed in U/g of hemoglobin (Hb). Glutathione peroxidase (GSH-Px) was determined by a spectrophotometric method based on the use of Ellman's Reagent [20]. The result was expressed in nmol/min/g of hemoglobin (Hb). Glutathion reductase activity was measured by the rate of NADPH oxidation to NADP⁺ which is accompanied by a decrease in absorbance at 340 nm (Kit, Cayman, USA). One unit of enzyme reduces 1μmol-oxidized glutathione per min at pH 7 at 25°C. Glutathion-S-transferase (GST) activity was evaluated according to Habig [21] by using CDNB (chloro-2, 4 dinitrobenzene) as the substrate at 340nm; results were expressed as nmol/min/g Hb.

Statistical analysis

Data are expressed as the Mean ± SEM. Statistics were performed with one-way variance (ANOVA) followed by Tukey test and the level of significance was set at $p < 0.05$. The data analysis was carried out by using IBM SPSS Statistics (v. 20).

RESULTS

Analysis of pectin

Our results showed that the content of the galactouronic acid in pectin of date was 51%, and the degree of esterification was 40, 1% (low esterified pectin).

Lead acetate burden

Lead acetate exposure in rat significantly ($p < 0.001$) augmented the lead burden in blood, when compared with untreated rat (C1, + 89.7%) and (C2, + 84.57%) respectively. Intake of pectin extract of date resulted in a significant ($p < 0.01$) depletion of lead content in blood, in comparison to lead acetate treated animals (Pb2), the decrease percentage is -20.03% (Table1).

Table 1. Effect of pectin against lead acetate-induced changes on lead burden, δ-ALAD activitie and erythrocyte-TBARS level in rats.

	4 weeks		8 weeks		
	C1	Pb1	C2	Pb2	Pb+P
Blood lead level (μg/dL)	0,02±0,006	0,15±0,005 ^a	0,02±0,003	0,11±0,001 ^c	0,09±0,001 ^d
δ-ALAD (μ mol PBG/min/L)	10,64±0,74	2,77±0,210 ^a	12,84±0,699	2,30±0,100 ^c	5,98±0,352 ^d
Erythrocyte-TBARS (μmol/L)	3,93±0,626	47,63±4,772 ^a	5,39±0,904	62,10±7,735 ^c	8,44±1,823 ^d

Values are Means ± SEM; n = 8. $p < 0.05$ by the tukey test. ^a versus control of 4 weeks, ^b versus lead exposed animanls of 4 weeks, ^c versus control of 8 weeks, ^d versus lead exposed animals of 8 weeks.

δ-ALAD: δ-aminolevulinic acid dehydratase; Erythrocyte-TBARS: Erythrocyte-Thiobarbituric acid reactive substances.

Hematological parameters

Table (2) summarizes mean values of hematological parameters of different groups. The results showed a significant ($p < 0.001$) decreased in RBC (-29.91%, - 4.84%), Hb (-41.85%, -25.12%), Ht (-26.94%, -18.3%), MCH (-15.35%, -25.26%), MCHC (-11.95%, -5.01%) and a significant increase in WBC (+ 86%, + 72.36%) in the lead acetate treated animals (Pb1 and Pb2), in comparison with control animals (C1 and C2) respectively, while MCV decreased non –significantly in lead acetate treated groups, when compared with controls animals.

Table 2. Effect of pectin against lead acetate-induced changes in some hematological variables in rat.

	4 weeks		8 weeks		
	C1	Pb 1	C2	Pb 2	Pb+P
RBC ($\times 10^6 \text{ mm}^{-3}$)	8,42 \pm 0,02	5,90 \pm 0,091 ^a	8,43 \pm 0,101	8,02 \pm 0,013 ^{c,b}	8,49 \pm 0,084 ^d
Hb (g/dL)	16,60 \pm 0,132	9,72 \pm 0,020 ^a	16,99 \pm 0,219	13,58 \pm 0,036 ^{c,b}	15,86 \pm 0,036 ^d
Ht (%)	43,57 \pm 0,169	31,83 \pm 0,386 ^a	44,96 \pm 0,703	36,73 \pm 0,263 ^{c,b}	41,66 \pm 0,342 ^d
MCV (fL)	52,25 \pm 0,046	50,36 \pm 1,32	55,71 \pm 0,632	42,83 \pm 0,115 ^c	45,45 \pm 0,673
MCH (pg)	20,08 \pm 0,085	17,00 \pm 0,067 ^a	21,42 \pm 0,164	16,01 \pm 0,143 ^c	30,84 \pm 0,125 ^d
MCHC (g/dL)	38,46 \pm 0,25	33,86 \pm 0,453 ^a	39,51 \pm 0,523	37,41 \pm 0,246 ^{c,b}	37,5 \pm 0,840
WBC ($\times 10^3 \text{ mm}^{-3}$)	3,88 \pm 0,02	27,73 \pm 1,545 ^a	3,84 \pm 0,005	13,90 \pm 1,46 ^{c,b}	3,88 \pm 0,004 ^d

Values are Means \pm SEM; n = 8. $p < 0.05$ by the tukey test. ^a versus control of 4 weeks, ^b versus lead exposed animals of 4 weeks, ^c versus control of 8 weeks, ^d versus lead exposed animals of 8 weeks.

RBC: red blood cells count; WBC: white blood cells count; Hb: hemoglobin concentration; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

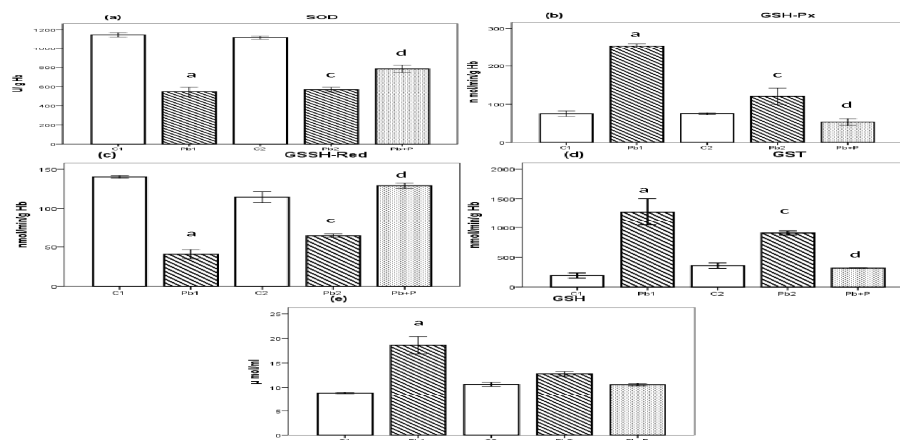
Pectin extract of date significantly elevated the RBC (+ 5.5%, $p < 0.01$), Hb (+ 14.35%, $p < 0.001$), Ht (+11.85%, $p < 0.001$), MCH (+11.29%, $p < 0.001$) when compared with lead acetate treated group (Pb2). No alteration in MCHC, WBC decreased significantly (-72.09%, $p < 0.001$) and insignificantly increased in MCV when treated with pectin of date.

ALAD activity

The effect of treatments on δ -ALAD activity is shown in Table 1. There was a significant decrease ($P < 0.001$) in activity of δ -ALAD in the intoxicated groups (Pb1 and Pb2) when compared to the control (C1 and C2) groups, the decrease percentage is -73.95% and -82.09% respectively. When, δ -ALAD activity was significantly increased (+ 61.5%, $p < 0.001$) after pectin treatment compared to intoxicated group.

Erythrocyte oxidative damage

Table 1 showed a significant ($p < 0.001$) elevation in the level of TBA-reactive product in erythrocyte (+ 91.73%, + 91.32%) in the intoxicated groups when compared to the control groups. A significant decrease ($p < 0.001$) in SOD (-52.36%, - 49.29%) and GSSH-Red activities (-70.98%, -43.91%) were evident in rat ingesting lead acetate (Pb 1 and Pb 2) in comparison to control groups (C1 and C2) respectively (Figure 1).

**Figure 1.** Effect of pectin against lead acetate-induced alteration on antioxidant enzyme activities and molecule in the erythrocytes of rats.

The data are expressed as mean \pm SEM. Significant differences set at $p < 0.05$. ^a versus control of 4 weeks, ^b versus lead exposed animals of 4 weeks, ^c versus control of 8 weeks, ^d versus lead exposed animals of 8 weeks.

(a)SOD :superoxide dismutase; (b) GSH-Px : Glutathione peroxidase; (c) GSSH-Red : Glutathione reductase; (d) GST : Glutathion-S-transferase; (e) GSH: Reduced glutathione.

Figure 1 showed that lead induced a significant ($p < 0.001$) increase in GSH-Px (+70.52%) and GST (+84.82%) levels. However, lead in Pb2 group revealed an insignificant increase in GSH-Px activity and significant increase ($p < 0.05$) in GST activity (+60.50%), in comparison to control group (C1). Whereas, GSH content significantly (+52.55%, $p < 0.001$) increased in erythrocytes of 4 week-lead acetate treated rats, but remains unchanged in the second period of the study.

Oral administration of pectin at dose of 3% significantly decreased the lipid peroxidation level as compared to lead acetate exposed group (Pb2) in red blood cell (-86.42%, $p < 0.001$) (Table 1). While administration of same dose significantly enhanced the SOD activity (+27.61%, $p < 0.01$) and GSH-Red activity (+50.26%, $p < 0.001$), as compared to lead acetate values (Pb2). GSH-Px and GST activities decreased significantly (-55.90%, $p < 0.001$ and -64.92%, $p < 0.05$ respectively) in-group received the pectin, when compared with lead acetate exposed animals (Pb2). Moreover, intake of pectin extract resulted insignificant reduction in GSH content, compared to intoxicated rat. (Pb2) (Figure1).

DISCUSSION

Lead is known to induce a broad range of physiological, biochemical, and behavioral dysfunctions in laboratory animals and humans. Its toxicity affecting the nervous systems, blood, cardiovascular system, kidneys, liver, gastrointestinal tract, and reproductive systems [22]. Our results indicated that lead induced erythrocytes oxidative damage and held hematotoxic in rats. The alterations in hematological changes serve as the earliest indicators of toxic effects on tissue. Following oral exposure to lead increased the lead level in blood, this reason, perhaps explain absorbed lead via blood to tissues, about 99% of blood lead is remain in the red blood cells (RBC) [23].

This study showed a decrease in the Hb level, Ht and RBC in lead intoxicated rat. In fact, lead toxicity induced anemia which may be attributed to the shortened life span of red blood cell, increased membrane fragility, increased erythrocyte destruction in hemopoietic organs, affection of hematogenesis by inhibited production of RBC from pro-erythroblast, decreased hemoglobin biosynthesis, or combination of all these factors[24]. The present findings are almost similar to previous studies [7, 25, 26, 27].

In contrary to this data, Golalipour [28] found that subchronic lead intoxication caused a slight increase in the red blood cell count, Hb and Ht. They suggested that tissue hypoxia is a possible mechanism for high production of RBCs in moderate lead poisoning. Nevertheless, lead could degenerate the lipid component and increased phosphatidylserine exposure (PS) on erythrocytes surfaces [29], by inhibition flippase, a key aminophospholipid translocase for the maintenance of PS asymmetry [30]. In addition, PS exposed to surfaces of cells is recognized and undergo a rapid phagocytosis by macrophages [29], which takes place mainly in the spleen, but also in the liver, a process called erythrophagocytosis [31], and as a result, the affected erythrocytes could be removed in blood stream leading to the apparition of anemia after lead poisoning [29]. Jang [30], showed that the oral administration of lead for 4weeks in the rats, induced a reduction of Ht, Hb level and increased spleen weight were observed along with enhanced splenic sequestration of red blood cells, this data are in agreement also with those of Kempe [32] who demonstrated that lead activates K^+ channels, leading to K^+ decline and erythrocyte shrinkage, then favoring phosphatidylserine exposure of the cell membrane. Therefore, the increase in erythrocyte destruction might due to inhibition of pyrimidine 5-nucleotidase by Pb^{2+} ions resulting in an accumulation of pyrimidine nucleotides in the erythrocyte, the accumulation of nucleotide affect red cell membrane by alteration of cellular energetic [33] and induced hemolytic anemia [30].

Our results revealed that lead induced a higher significant increase of WBC after 4 weeks. This was in accordance with the study of Shah [34] and Al-Ali [35] who illustrated acute and chronic feeding of lead nitrate resulted in the enhancement of total leucocytes count. Moreover, Pb^{2+} ions increased WBC in automobile workers exposed to lead when compared to the controls [26]. In contrary to this data, animals exposed to 50 mg/kg /day of lead have a significant decline in total leukocyte count, lymphocyte and monocyte content [7]. In addition, the study of Suradkar [36] revealed a leucopenia and lymphopenia in intoxicated rats. Indeed, Ray [37] concluded that lead caused in mice leucopenia, they suggested that lead might exert a cytotoxic effect on both lymphopoietic and myelopoietic tissues. At the same time, our findings showed that administration of lead at 350mg/kg for one month induced a significant decrease of MCH, MCHC and insignificant for MCV. These results agreed with the finding obtained by Yilmaz [27] and Golalipour [28]; they proved that lead induced a decrease in MCV and MCH.

Our data showed a decrease in hemoglobin concentration, which may result from a decreased of hemoglobin synthesis [35], that may be result also from decreased of enzymes involved in heme biosynthesis. In fact, the most sensitive enzyme to the toxic effects of lead is probably δ -aminolevulinic acid dehydratase (δ -ALAD), which catalysis the formation of porphobilinogen (PBG) from condensation of two units of delta-aminolevulinic acid (δ -ALA) [38]. Oral exposure to lead acetate in male rat induced a significant decrease of δ -ALAD activity when

compared with control groups. This results are in agreement with those of Santos [39] and Zareba [40] who found a reduction of δ -ALAD activity in erythrocytes, liver and bone marrow in rats and rabbits. Furthermore, the results of Dongre [26] and Arun [41] indicated that lead inhibited the activity of δ -ALAD enzyme in automobile workers, because they observed that the ratio of activated/non-activated δ -ALAD was significantly increased. In addition, Gurer-Orhan [42] found a significant correlation between ALAD activity and blood lead levels in human subjects. The accumulation of δ -aminolevulinic acid (ALA) upon exposure to lead induced generation of ROS and resulted oxidative stress [38]. A possible mechanism for lead toxicity is the formation of lead-sulfhydryl complexes [33]. δ -ALAD is inhibited by lead via direct binding of lead to the SH groups that are essential for the catalytic activity of the enzyme [43].

Lead produced reactive oxygen species (ROS) such as hydrogen peroxide, superoxide ion, singlet oxygen and hydroxyl radical [25], that result in DNA damage, depletion of cell antioxidant defense systems and lipid peroxidation (TBARS) in tissue and blood [44]. Our current investigations showed a significant increased concentration of LPO in erythrocytes after an exposure to lead acetate in intoxicated groups when compared to control groups, and exhibited that lead promoted oxidative damage in erythrocytes of rats. Our results are in agreement with results obtained by Emrah [25]. Lipid peroxidation caused cell death by disruption of cell membrane integrity [45]. Arun [41] they showed a positive correlation between blood lead level and serum MDA concentration in exposed group. They suggested that increase production of lipid peroxide (MDA) in workers groups may be due to rise of ALA in blood after lead exposure.

The findings of our study showed that lead acetate induced a significant decrease of erythrocyte-SOD activities, erythrocyte-GSSH reductase activities, and erythrocyte-GSH level, while, the activity of GSH-Px and GST were increased in red blood cells of exposed rats when compared to control groups. This data is similar to the study of Emrah [25], who found a decrease under control values in erythrocyte SOD in the rat feeding lead, they suggested that SOD level of red blood cells in peripheral or bone marrow also might increase to compensate lead induced enzyme inhibition. The study of Sharma [7], illustrated that lead nitrate reduced the activities of SOD and GSH level. The alteration of this antioxidant activities by lead may be due to inhibit functional SH groups [46], or induces copper deficiency resulted in decreased erythrocyte SOD activity [47]. Our results showed also that lead induced an increase of GST activities; this enzyme plays an important role on detoxification and elimination of toxins. It has been known that glutathione S-transferases (GSTs) can reduce lipid hydroperoxides through their Se-independent glutathione peroxidase activity and that these enzymes can also detoxify lipid peroxidation end products such as 4-hydroxynonenal (4-HNE) [48]. Recent studies suggesting that the GSTs provide a formidable defense against oxidative stress [48], this data confirmed by [49] who showed that the lipid peroxidation products, particularly hydroperoxides and 4-HNE, are involved in the mechanisms of stress-mediated signaling and that it can be modulated by the Alpha class GSTs through the regulation of the intracellular concentrations of 4-HNE.

Oral administration of date pectin extract for one month increased significantly RBC and Hb level in rat treated group (Pb+P) when compared with Pb2 group. However, our data showed that discontinuation of lead exposure induced an increase of the both parameters. At the same time, pectin induced a significant increase of Ht and MCH, significant decreased of WBC and no modification of MCHC in rat feeding pectin of date when compared to intoxicated rats. In fact, the discontinuation of Pb^{2+} ions increased Ht, MCHC and WBC with no modification of MCH. Elsewhere, our data revealed that pectin enhanced ALAD activities, and decreased lipid peroxidation product. Indeed, we have noted a significant increase in erythrocyte-SOD and GSH activities in rat treated with pectin when compared to intoxicated group. Although, pectin decreased significantly GSH-Px, GST activities and non-significantly GSH level in RBC. In parallel, the arrest of lead exposure had not modify all this parameters. The study of González [50] on patients with lead poisoning, they found no significant reduction in blood Pb after a recovery period of about four months. The results obtained through this study showed the possible healing effects of pectin extract of date on lead induced erythrocytes oxidative damage. This showed that pectin could accelerate the removal of lead and reduce its adverse effects on red blood cells and hematological parameters. Furthermore, Our data showed that pectin of date had a degree of esterification less than 50% (40,1%), this is might explain the effect of pectin against hemotoxic action and oxidative stress of lead, by high affinity of low esterified pectin molecule for Pb^{2+} ions [51]. Previous studies have illustrated the relationship between the degree of methylation of pectin and metal binding, Serguschenko [52] proved that pectin exerts high metal binding activity regarding bivalent metal ions and Ankit [8] confirmed that carboxylic acid groups are active participants in Pb binding by citrus pectin.

In the present study, treatment of animal with low esterified pectin showed a significant decrease of lead blood level compared to Pb2 group, these results agreed with results obtained by El-Nahal [53] and Ouldali [54]. Furthermore, treatment with low-esterified pectin promoted the decrease in lead content in the liver, reduction of LPO activity, and recovery of lipid metabolism parameters [55]. In addition, Ostapenko [56] found that

MEDETOPECT (Commercial apple pectin product) is effective agent for prophylaxis of lead incorporation in industrial conditions. Pectin has permitted to increase the lead elimination from rat organism [57]. Beside, Zhao [58] reported the safe and effective use of pectin as a chelating agent. Previous study of Dongowski [59] concluded that application of oligogalacturonic acids enhanced lead elimination through blood and organs. Moreover, addition of rhamnogalacturonic parts of pectin into the lead-enriched diet in rats contributed to slow absorption of lead in rats and increased its excretion in feces. In contrary to this, lead complexed by dRGII in fruits, vegetables and juice is mostly unavailable for intestinal absorption. However, the addition of dRGII after chronic lead exposure in rats does not help lead detoxification [60].

Moreover, the study of Sergey [61] reported that low DM pectin has been shown to penetrate through the gastrointestinal mucosa, and it has a higher probability of reaching the epithelium as compared with HM pectin. The oligogalacturonic and short-chain fatty are the products of cleavage of pectin by fermentation, these products are absorbed into blood [6]. Therefore, treatment of rats with low esterified pectin significantly reduced lead retention in tissues; these results suggest that pectin with a low degree of esterification may be considered as perspective dietary compounds removing environmental lead from the body [62].

Conclusion

In conclusion, many chelating agents are currently used to removal lead toxicity, such as pectin, the data suggests oral administration low-esterified pectin reduced oxidative stress in erythrocyte and normalized the hematological parameters. Pectin had the ability to chelate lead and subsequently acts as active natural compound to discharge lead contamination.

Conflict of Interest

All authors declare no conflicts of interest.

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