Slov Vet Res 2022; 59 (4): 195–209
DOI 10.26873/SVR-1591-2022
UDC 615.9:546.81:616-091.8:615.035:547.458.88:599.323.452

Original Research Article

PECTIN IMPROVES HEMATO-BIOCHEMICAL PARAMETER, HISTOPATHOLOGY, OXIDATIVE STRESS BIOMARKERS, CYTOKINES AND EXPRESSION OF HEPCIDIN GENE IN LEAD INDUCED TOXICITY IN RATS

Sabry M. El-Bahr[1,2]*, Saad Al-Sultan[1,3], Ahlam F. Hamouda[4], Shimaa. A. E. Atwa[5], Seham Y. Abo kora[6], Aziza A. Amin[7], Saad Shousha[1,8], Sameer Alhojaily[1], Aymmen Alnehas[9], Rabab R. Elzogby[10]

[1]Department of Biomedical Sciences, [3]Department of Public Health, [9]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia, [2]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, 21523, Egypt, [4]Department of Forensic Medicine and Toxicology, Teaching Hospital, [5]Department of Biochemistry, [6]Department of Pharmacology, [7]Department of Histopathology, [8]Department of Physiology, Faculty of Veterinary Medicine, Benha University, Benha, 13736, [10]Department of Pharmacology, Faculty of Veterinary Medicine, NewVally University, Egypt

*Corresponding author, E-mail: selbahar@kfu.edu.sa

Abstract: Publications concerning the protective effect of pectin against lead induced toxicity in rats are not available. In order to study such effect, 40 male rats were divided into 3 groups. The first group was contained 10 rats that kept as control group. The second group was contained 10 rats that received pectin at dose of 100 mg/kg BW during experimental period (8 weeks). The third group was contained 20 rats that received 400mg/kg BW of lead acetate daily for 4 weeks then divided into two subgroups (3A and 3B). Subgroup 3A contained 10 rats that still receive lead acetate in the same dosage whereas, subgroup B co-treated with lead acetate and pectin daily for another 4 weeks. Blood samples were collected after 2, 4 and 8 weeks from the start of the experiment. Liver, kidney and bone marrows were collected only at the end of the experiment. Lead acetate induced anemia only after 4 weeks of administration as reflected on decreased values of Hb, PCV, MCV, MCH and MCHC. These indices remained at lower levels in lead acetate treated groups until the end of the experiment. Concentrations of serum ferritin, iron, total antioxidant capacity (TAC) and reduced glutathione (GSH) and the expression of hepatic hepcidin gene were decreased significantly in lead acetate intoxicated rats compared to control. Activities of ALT and AST and concentrations of urea, creatinine, Nitric oxide (NO), TNF-α, IL-6, total iron binding capacity (TIBC) and lead were increased significantly in lead acetate intoxicated group compared to control. Hepatic degeneration and hemorrhage, renal lytic necrosis and apoptosis of myeloid cells were most prominent changes in lead intoxicated rats. Lead acetated related changes were improved by co-treatment with pectin however; normal control values have not been achieved. Conclusively, pectin is recommended to protect against lead acetate toxicity in rats.

Key words: lead acetate; toxicity; pectin; hepcidin; oxidative stress biomarkers; histopathology

Introduction

One of the most serious environmental medicine issues is lead poisoning. Lead contamination in the environment caused by industrial lead production and metal recycling (1). Due to its harmful cumulative action in the environment, lead can affect all biological systems by exposure from multiple sources such as air, water, and food. Lead has the ability to migrate up the food chain, causing harm to humans and other animals. Lead induced microcytic hypochromic anemia in mammals due to its interaction with iron and copper metabolism (2). Lead may disrupt the integrity of the RBC membrane, making it more fragile, resulting in a disorder of red blood cell metabolism in the bone marrow or mature erythrocytes, inhibit the enzyme ferrochelatase and reducing iron (Fe) incorporation into heme

Received: 22 September 2022
Accepted for publication: 13 October 2022

and disrupting heme synthesis (3). Lead exposure has been observed to reduce serum iron and transferrin saturation levels in rats (4). Lead acetate has hepatoxic effect which increase hepatocyte permeability in rats. The damaged hepatocyte cell membrane of hepatocytes leading to escape of liver enzymes to blood. Lead toxicity produces an increase in cellular basal metabolic rate, irritability, and destructive alteration of liver cells due to its oxidative effect (5). Oral dose of lead acetate caused a significant rise in blood urea and serum creatinine in rats (6). The main mechanism responsible for lead toxicity is oxidative stress. This type of stress causes changes in the composition of fatty acids in cells membrane (affecting processes such as exocytosis and endocytosis, as well as signal transduction processes). The production of reactive oxygen species (ROS), the activation of lipid peroxidation, and the depletion of antioxidant reserves are all factors that contribute to lead exposure (3, 7). Hepcidin gene expression is reduced after experimentally induced anemia and hypoxia, which could explain the increased (Fe) release from reticuloendothelial cells and higher (Fe) absorption normally observed in these conditions, suggesting hepcidin involvement in anemic states. Lead has also been found to prevent (Fe) from being transferred from endosomes to the cytoplasm (8). Hepcidin expression was found to be lower in people with anemia of chronic disorders (ACD) and in mammalian models that resembled ACD (9, 10). In patient and mammals with (ACD), iron deficiency anemia, serum hepcidin levels and/or liver mRNA expression were both decreased considerably (9). Additionally, Hypoxia may diminish hepcidin expression while increasing serum iron and transferrin saturation, allowing massive erythropoiesis to compensate for tissue hypoxia (11). Pectin is a galacturonic acid polymer found mostly in plant walls. It can be isolated from fruit pips, apple pulp, and peal (12). Pectin rich in galacturonic acid (GalA) are effective at chelating heavy metals (13). Pectin's ability to chelate metals in the digestive system and inhibit absorption while aiding their removal in the faces, toxic metal absorption and bioaccumulation were reduced with its administration (14). Oral administration of pectin resulted in decrease lead absorption (15). In industrial settings, commercial apple pectin is an excellent agent for preventing lead incorporation (16). The goal of this work was to evaluate the effect of pectin on hemato-biochemical parameter, histopathology, oxidative stress biomarkers, cytokines and expression of Hepcidin gene in lead intoxicated rats.

Materials and methods

Chemicals

Lead acetate obtained from Al Gomhoria company, Egypt. Pectin obtained from Sigma Company, Egypt.

Experimental animals and design

This experiment was carried out according to the guidelines of the Institutional Animal Ethics Committee, Benha University, Egypt, and Approval (Permission # BUFVTM 05-12-21**).** Forty male albino rats were purchased from Lab Animal House at Vet College Benha University; their average weight was (160±10g). They kept in well-ventilated metal cages throughout the study and acclimatized for one week at a temperature of 18-24°C with 12 hours of light and darkness, on normal feed diet and water ad libitum. The experimental design illustrated obviously at Table 1. This design showed that, 40 male rats were divided into 3 groups. The first group was contained 10 rats that kept as control group and received normal physiological saline daily during experimental period (8 weeks). The second group was contained 10 rats that received pectin at dose of 100 mg/kg BW (17) during experimental period (8 weeks). The third group was contained 20 rats that received 400mg/kg BW of lead acetate daily (18) for 4 weeks then divided into two subgroups (3A and 3B). Subgroup 3A contained 10 rats that still receive lead acetate in the same dosage whereas, subgroup B co-treated with lead acetate and pectin daily for another 4 weeks. Blood samples were collected after 2, 4 and 8 weeks from the start of the experiment. The whole blood was used or the detection of hematological indices (Hb, PCV, MCV, MCH and MCHC). The obtained serum was used for the estimation of the activity of ALT and AST and the concentration of urea, creatinine, ferritin, iron, TIBC TAC, GSH, NO, TNF-α, IL-6 and lead. Liver, kidney and bone marrows collected only at the end of the experiment and subjected to histopathological examination. Portion of liver tissues was frozen by liquid nitrogen until used for detection of expression of hepatic hepcidin gene.

Table 1: The experimental design of the study

Time	Samples	Parameters measured	Groups			
			Group 1	Group 2	Group 3	
After 2 weeks	blood	Hb, RBCs, PCV, MCV, MCH, MCHC	✓	✓	✓	
After 4 weeks	blood	Hb, RBCs, PCV, MCV, MCH, MCHC,	✓	✓	✓	
					3A	3B
After 8 weeks	Blood, serum, liver, kidney, bone marrow	Hb, RBCs, PCV, MCV, MCH, MCHC, Basophilic Stippling cell, Ferritin, Iron, TIBC, AST, ALT, urea, creatinine, TAC, GSH, NO, TNF-α, IL-6, lead, Histopathology	✓	✓	✓	✓

Assessment of Complete blood count

Assessing of complete blood count was performed using an electronic cell counter (VetScan HM5 Hematology system, Abaxis, Inc., Union City, CA, USA).

Assessment of liver and kidney function tests and iron profile

Activities of ALT and AST were performed as described earlier (19). Serum urea and creatinine were performed as described previously (20, 21), respectively. Serum iron, ferritin and Total iron Binding Capacity (TBIC) were performed as described earlier (22, 23, 24), respectively.

Assessment of serum oxidative stress biomarker and cytokines concentrations

The total antioxidant capacity (TAC), reduced glutathione (GSH) and nitric oxide (NO) were performed as described in previous researches (25, 26, 27), respectively. TNF-α and IL-6 concentrations were determined by using ELISA kit that described earlier (28).

Detection of lead residues in blood

Detection of lead residues in blood was performed as described earlier (29). Briefly, 1ml whole blood was measured into clean test tubes, followed by 1 ml concentrated nitric acid containing 0.1 percent triton-100, which was mixed carefully. Cotton wool was used to plug the test tubes, which were then placed on the bench overnight. The mixture was then cooked in a water bath at 100°C for 20 minutes on the second day, and then allowed to cool. The digested blood samples were transferred to a measuring cylinder and filled with distilled water to a volume of 25 ml. Lead residue was determined by using Perkin-Elmer 2380 Atomic absorption spectrophotometer.

Investigation of mRNA expression of Hepcidin gene

This stage was completed at Benha University's Central Laboratory, Faculty of Veterinary Medicine. The following primer sets were used to dissect liver samples from all rat groups (30). Hepcidin, sense (5 - GAAGGCAAGATGGCACTAAGCA -3') and anti-sense (5 - TCTCGTCTGTTGCCGGAGATAG -3), and actin as a housekeepin gene, sense (5 AGAAGAGCTATGAGCTGCCTGACGCG-3) and anti-sense (5'-CTTCTGCATCCTGTCAGCGATGC-3('.

The Blood Smear (Field stain)

Blood smears are used to look at single-cell spread in blood components (31).

Histopathological examination

Specimens were taken immediately from liver and kidneys of all groups, fixed in 10% buffered neutral formalin for 24 hours. After proper fixation, the specimens were washed in running tape water, dehydrated in different grades of ethyl alcohol, cleared in xylol and embedded in paraffin, then blocked and sectioned as 5 mm thickness. Then stained by hematoxylin and eosin and examined microscopically (32). Pathological alterations were examined using an Olympus light microscope, femur bones were collected, decalcified, fixed and samples were processed (33). All pathological markers were measured using a standard semi quantitative scoring approach to compare the severity of lesion severity between groups. The following is a five-

point ordinal scale: (0) no changes, (1) mild 25 percent tissue damage, (2) moderate 25 percent: 50 percent tissue damage, (3) severe 50 percent: 75 percent tissue damage, and (4) extensive severe >75 percent tissue damage (34).

Statistical analysis

The data was analyzed using SPSS 15.0 statistical software and provided as mean SD (SPSS Inc., Chicago, IL). One analysis of variance (ANOVA) was used for statistical analysis of the current data.

Results

Complete blood count

The mean and standard deviation values of complete blood count of the different experimental groups after 2, 4 and 8 weeks were depicted in Table 1, 2 and 3, respectively. After 2 weeks from the start of the experiment, all measured hematological indices (Hb, PCV, MCV, MCH and MCHC) remained unchanged significantly in both pectin and lead acetate treated groups compared to the control (Table 2). However, after 4 weeks from the start of the experiment, HB, RBCs, PCV, MCH, MCHC values were decreased significantly in lead acetated treated group compared to control (Table 3). These values remained unchanged significantly in pectin treated rats compared to control (Table 3). After 8 weeks from the start of the experiment, all hematological indices were decreased significantly in lead acetate rats (group 3A) compared to control (Table 4). In addition, basophilic stippling cell noticed in this compared to control (Table 4). Co-treatment of rats with lead acetate and pectin recovered all measured hematological indices into normal control values except for MCV and MCHC that remained lower than that of control values (Table 4). Furthermore, basophilic stippling cell was disappeared in rats co-treated with lead acetate and pectin compared to control (Table 4).

Group 1: contained 10 rats that kept as control group. Group 2: contained 10 rats that received pectin at dose of 100 mg/kg BW during experimental period (8 weeks). Group 3: contained 20 rats that received 400mg/kg BW of lead acetate daily for 4 weeks then divided into two subgroups (3A and 3B). Subgroup 3A: contained 10 rats that still receive lead acetate in the same dosage. Subgroup B: contained 10 rats that co-treated with lead acetate and pectin daily for another 4 weeks.

Table 2: effect of lead administration on blood indices in different experimental groups after 2 weeks from the start of the experiment

Parameters	Groups		
	Group 1	Group 2	Group 3
Hb (mg/dl)	14.18±0.52^{a}	12.15±2.41^{a}	12.30±3.70^{a}
RBCs (×10^{6} /µml)	6.71±0.20^{a}	6.32±1.30^{a}	6.42±1.15^{a}
Pcv (%)	36.60±2.35^{a}	37.40±2.24^{a}	39.09±7.54^{a}
MCV (fl/cell)	60.5±1.15^{a}	62.1±2.05^{a}	60.62±2.04^{a}
MCH (pg/cell)	20.43±0.38^{ab}	21.34±2.24^{a}	16.09±4.41^{a}
MCHC (g/dl)	33.90±1.22^{b}	32.80±1.18^{a}	34.77±1.50^{a}

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P ≤ 0.05).

Table 3: effect of lead administration on blood indices in different experimental groups after 4 weeks from the start of the experiment.

Parameters	Groups		
	Group 1	Group 2	Group 3
Hb (mg/dl)	14.03±0.30^{a}	13.07±0.51^{a}	9.64±0.07^{b}
RBCs (×10^{6} /µml)	6.83±0.06^{a}	6.99±0.12^{a}	5.12±.0.08^{b}
PCV (%)	34.66±2.52^{a}	33.87±2.49^{a}	26.60±0.2^{b}
MCV (fl/cell)	53.71±1.48^{a}	52.69±1.60^{a}	45.94±2.49^{b}
MCH (pg/cell)	20.97±0.44^{a}	19.88±1.50^{a}	16.01±0.71^{b}
MCHC (g/dl)	38.28±0.37^{a}	36.40±2.40^{a}	31.89±0.49^{b}

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P ≤ 0.05).

Table 4: Effect of lead acetate on blood indices in different experimental groups after 8 weeks from the start of the experiment

Parameters	Groups			
	Group 1	Group 2	Group 3	
			3A	3B
Hb (mg/dl)	14 ±0.95[a]	13.45 ±1.67[a]	9.01±0.11[c]	13.3± 0.55[a]
RBCs (×106/µml)	6.00± 0.6[a]	5.88± 0.68[a]	4.50± 0.13[b]	6.22± 0.34[ab]
PCV (%)	35.63± 2.41[a]	32.99± 2.37[a]	28.29 ±0.77[c]	32.36 ±2.43[b]
MCV (fl/cell)	61.48± 1.20[a]	57.88± 1.29[a]	52.13± 0.27[b]	52.08±0.98[b]
MCH (pg/cell)	21.13± 0.4[a]	22.15± 0.51[a]	18.96±0.39[b]	21.26±0.35[a]
MCHC (g/dl)	35.89±1.19[a]	37.99±1.21[a]	31.68±0.51[b]	31.23±1.45[b]
Basophilic Stippling cell	-	-	+	-

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P ≤ 0.05).

Table 5: Effect of lead acetate on liver and kidney function tests and iron profile in different experimental groups

Parameters	Groups			
	Group 1	Group 2	Group 3	
			group A	group B
AST (U/ml)	30.10± 3.18[c]	32.21± 2.87[c]	145.62± 4.10[a]	103.7± 1.53[b]
ALT (U/ml)	29.8 ±1.71[c]	31.66 ±2.01[c]	59.33± 0.88[a]	35.91± 3.00[b]
Urea (mg/dl)	25.98± 1.96[c]	27.79± 1.10[c]	131.7± 2.94[a]	42.30± 0.36[b]
Creatinine (mg/dl)	0.68± 0.02[c]	0.64± 0.05[c]	2.9±0.34[a]	0.83± 0.02[b]
Ferritin (ng/dl)	0.7±0.057[a]	0.81±0.07[a]	0.47±0.028[b]	0.70±0.054[a]
Iron (µg/dl)	2.31±0.089[a]	2.42±0.06[a]	1.29±0.10[b]	2.25±0.07[a]
TIBC (mcg/dl)	6.01±0.51[b]	6.06±0.49[b]	8.53±0.24[a]	6.87±0.53[b]

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P ≤ 0.05).

Table 6: Effect of lead acetate on oxidative markers, cytokines and lead in different experimental groups

Parameters	Group 1	Group 2	Group 3	
			3A	3B
TAC (µmol/L)	2.45± 0.22[a]	2.51± 0.30[a]	0.99± 0.10[c]	1.93± 0.10[b]
GSH (mg/g)	56.18 ±3.59[a]	55.22±3.60[a]	33.98±2.14[c]	41.45±1.73[b]
NO (µmol/L)	19.06±2.25[c]	20.10±2.40[c]	42.24±1.57[a]	30.11±1.05b[c]
TNF-α (pg/ml)	19.85±5.59[c]	21.99±5.64[c]	59.84 ±4.59[a]	47.45±4.07[b]
IL_6 (pg/ml)	9.87±0.12[c]	9.67±0.24[c]	45.72±1.67[a]	19.64±1.27[b]
Serum lead (mg/dl)	0.95±0.06[a]	0.98±0.07[a]	11.65±0.63[c]	5.95±1.54[b]

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P ≤ 0.05). TAC (total antioxidant capacity), GSH (reduced glutathione), NO (nitric oxide).

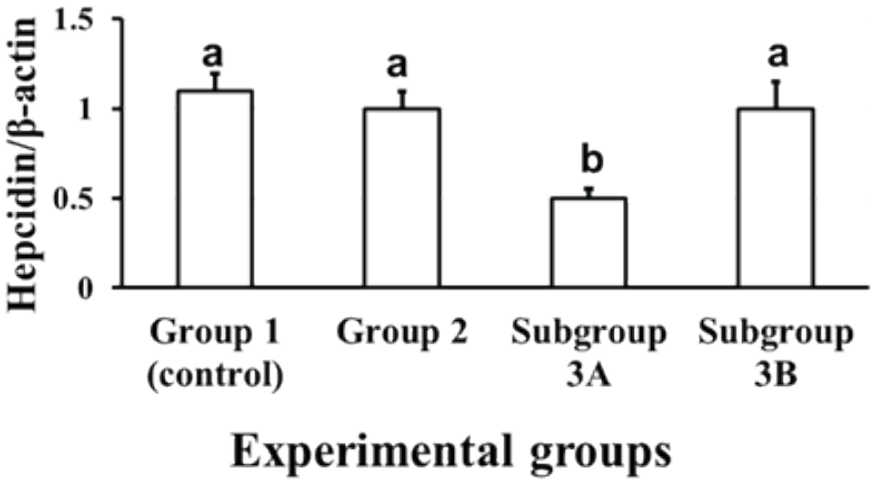

Figure 1: Effect of lead acetate on liver hepcidin mRNA expression level and ameliorative effect of Pectin in male albino rats

Table 7: The score of histopathological lesions in different experimental groups

Lesion score	Group 1	Group 2	Group 3	
			3A	3B
	Liver			
Congestion of hepatic blood vessels	0	0	4	1
Activation of Vonkupfer cell	0	0	3	0
Perivascular Leukocytic infiltration	0	0	3	0
Degenerative changes	0	0	4	1
Necrosis of hepatic cells	0	0	3	0
Nuclear changes	0	0	3	0
	Kidney			
Congestion of renal blood vessels	0	0	3	1
Degeneration of blood vessels wall	0	0	2	0
Perivascular edema	0	0	3	0
Tubular epithelial degeneration and necrosis	0	0	4	1
Hyaline and cellular casts	0	0	2	0
Interstitial leukocytic infiltration	0	0	3	0
necrosis of glomerular tuft	0	0	2	0
	Bone marrow			
Reduction in erythropoiesis	0	0	3	1
Degeneration of myeloid	0	0	2	0

Liver and kidney function tests and iron profile

Activities of ALT and AST and concentrations of urea, creatinine and total iron binding capacity (TIBC) were increased significantly in lead acetate intoxicated group (subgroup 3A) compared to control (Table 5). However, concentrations of serum ferritin and iron, were decreased significantly in lead acetate intoxicated rats (subgroup 3A) compared to control (Table 5). These parameters were improved in rats co-treated with lead acetate and pectin than that of lead acetate treated rats but the normal control values have not been achieved for ALT, AST, urea and creatinine (Table 5).

Oxidative stress biomarkers, cytokines and lead concentrations

The concentrations of NO, TNF-α, IL-6 and lead concentrations were increased significantly in lead acetate intoxicated group (subgroup 3A) compared

to control (Table 6). However, concentrations of serum TAC and GSH were decreased significantly in lead acetate intoxicated rats (subgroup 3A) compared to control (Table 6). These parameters were improved in rats co-treated with lead acetate and pectin than that of lead acetate treated rats but the normal control values have not been achieved (Table 6).

mRNA gene expression

The hepcidin mRNA gene expression of different experimental groups was illustrated in Figure 1. The expression of hepcidin gene in rats fed pectin alone remained unchanged significantly compared to the control (Fig. 1). The expression of hepcidin gene in liver tissue were decreased significantly in lead acetate intoxicated rats (subgroup 3A) compared to control (Fig. 1). Co-treatment of rats with lead acetate and pectin (subgroup B3) up-regulated the expression of this gene to normal control value (Fig. 1).

Histopathological examination

The score of lesions of histopathological examination were seen in the liver, kidneys, and bone marrow of rats in different experimental groups as shown in Table 7. The histopathoogical picture of rats tissues that received pectin only (group 2) were not showed in the current study because it looks like the control group (group 1) as illustrated in Table 7.

The histological examination of the liver in subgroup 3A showed that there were congestion of the hepatic blood arteries and blood sinusoids, as well as portal distension and leukocytic cellular infiltration, primarily lymphocytes and macrophages (figure 2A). Hepatocytes also showed significant degeneration in the form of diffuse hydropic degeneration (figure 2B) with diffuse hemorrhage in the hepatic parenchyma, as well as multifocal patches of coagulative necrosis with pyknotic nucleus. Furthermore, diffuse regions of lytic necrosis in the hepatic parenchyma were detected, that consisted of necrotic debris mixed with erythrocytes and leukocytes (figure 2C&D). The hepatic tissue collected from rats in subgroup 3B showed an improvement in the hepatocellular architecture when compared with rats in subgroup 3A. In comparison to the control, the liver tissue returned to its normal histological structure. The majority of the hepatic parenchyma had recovered, and only minor hepatocyte vacuolar degeneration (figure 2E) was observed, while the portal area appeared normal. The microscopic analysis of hepatic tissue from control and pectin treated groups showed normal histological structure and showed in the figure.

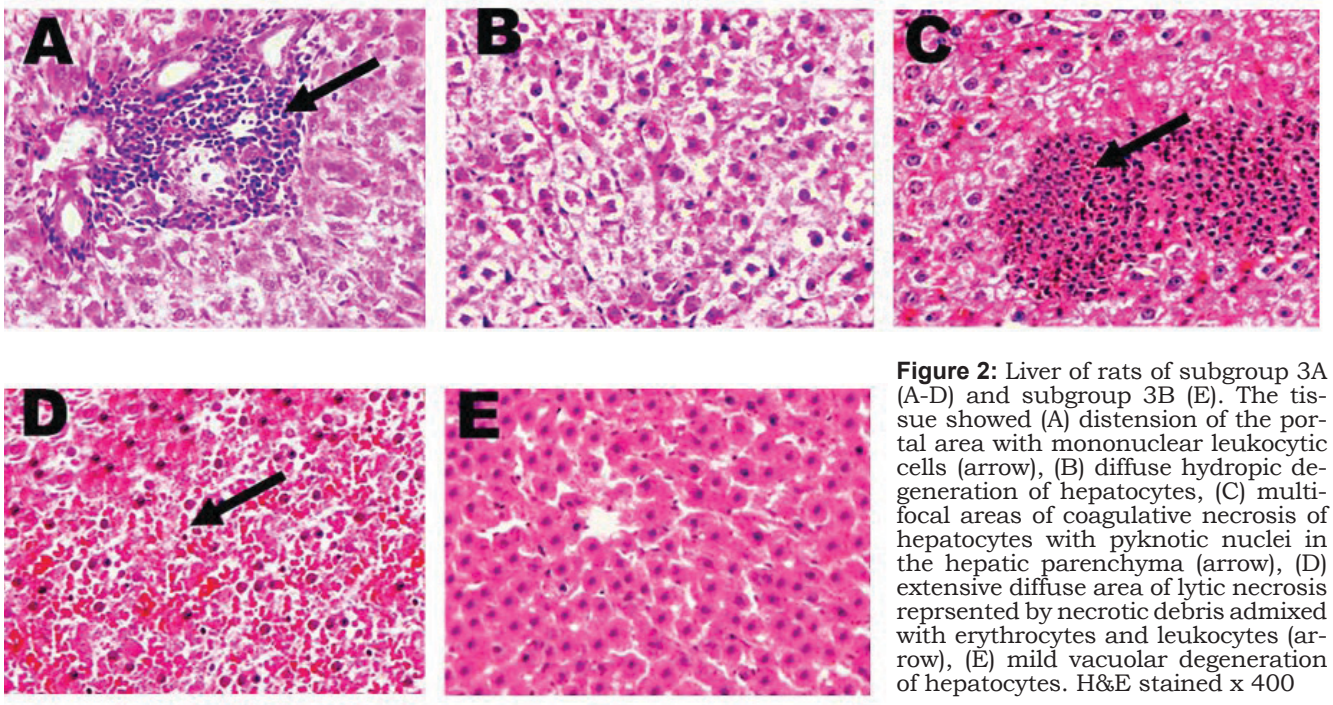

Figure 2: Liver of rats of subgroup 3A (A-D) and subgroup 3B (E). The tissue showed (A) distension of the portal area with mononuclear leukocytic cells (arrow), (B) diffuse hydropic degeneration of hepatocytes, (C) multifocal areas of coagulative necrosis of hepatocytes with pyknotic nuclei in the hepatic parenchyma (arrow), (D) extensive diffuse area of lytic necrosis reprsented by necrotic debris admixed with erythrocytes and leukocytes (arrow), (E) mild vacuolar degeneration of hepatocytes. H&E stained x 400

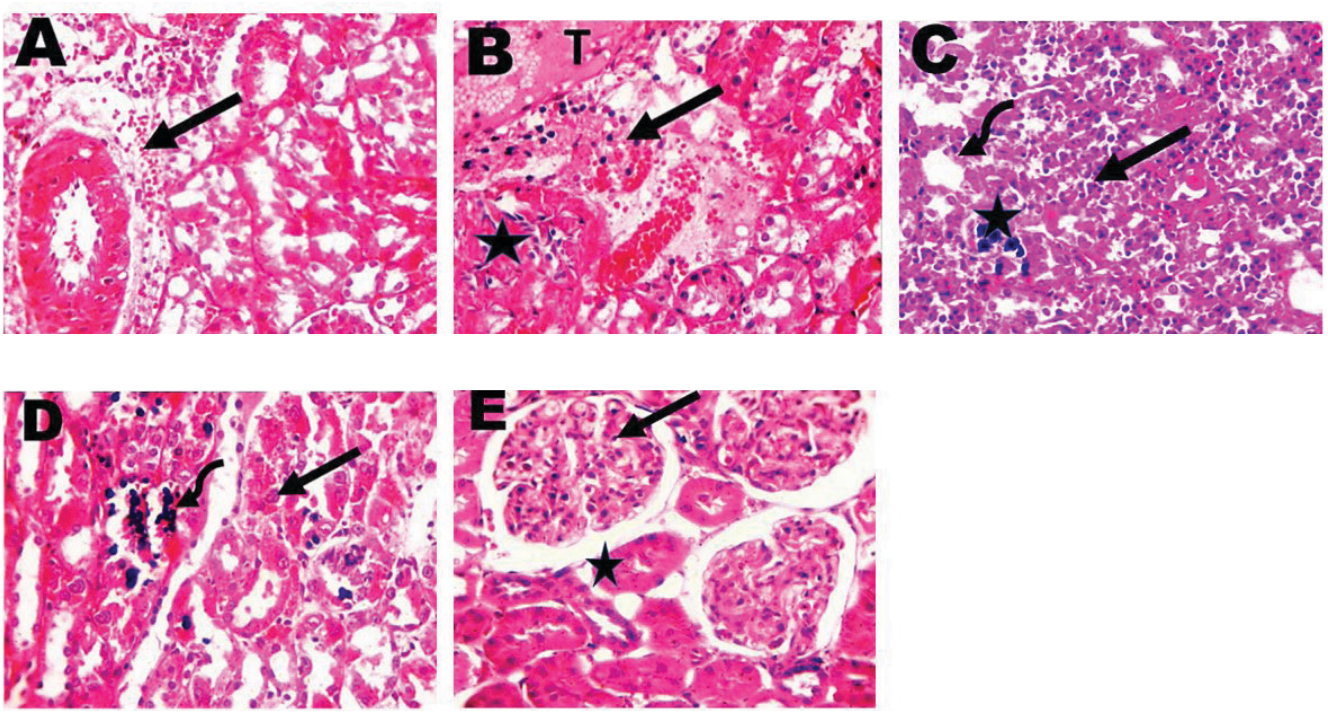

Figure 3: kidney of rats for subgroup 3A(A-D) and subgroup B (E). tissue showed (A) perivascular edema admixed with erythrocytes (arrow) with vacuolar degeneration of the lining epithelium of some renal tubules and its necrosis with pyknotic nuclei in other tubules, (B) necrosis of the glomerular tuft (asterisk) and with thrombosis of the renal blood vessels (T) with extensive focal area of lytic necrosis represented by necrotic debris admixed with erythrocytes and leukocytes (arrow), (C) necrosis of the lining epithelium of the con-voluted tubules (zigzag arrow), inter tubular mononuclear leukocytic cellular infiltrations (arrow) with precip-itation of lead pigment renal tubules (asterisk), (D) precipitation of lead pigment in the renal tubules of renal medulla (zigzag arrow) with the presence of intra-nuclear eosinophilic inclusion bodies (arrow), (E) mild vacuolation of the endothelial cell lining of glomerular tuf with cloudy swelling of the lining epithelium of some renal tubules. H&E stain x 400

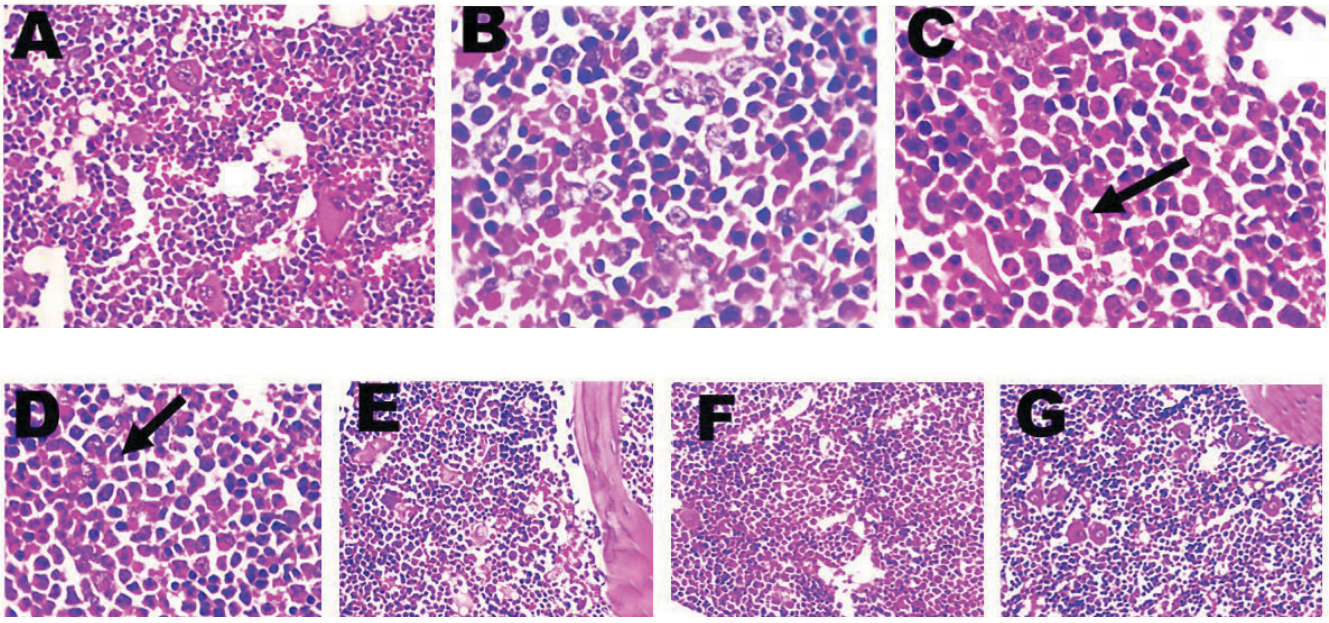

Figure 4: Bone marrow of rats for control (A-B), subgroup 3A (C-E) and subgroup 3B (F-G), showing (A-B) nor-mal bone marrow (A, x400, B, x1000), (C) degeneration and apoptosis of myeloid cells (arrow, x1000), (D) intra-nuclear eosinophilic inclusion bodies (arrow, x1000), (E) degeneration of megakaryocytes (x1000), (F) hyperplasia of megakaryocytes (x1000), (G) restoring of normal histological structure of bone marrow (H&E-stained x1000)

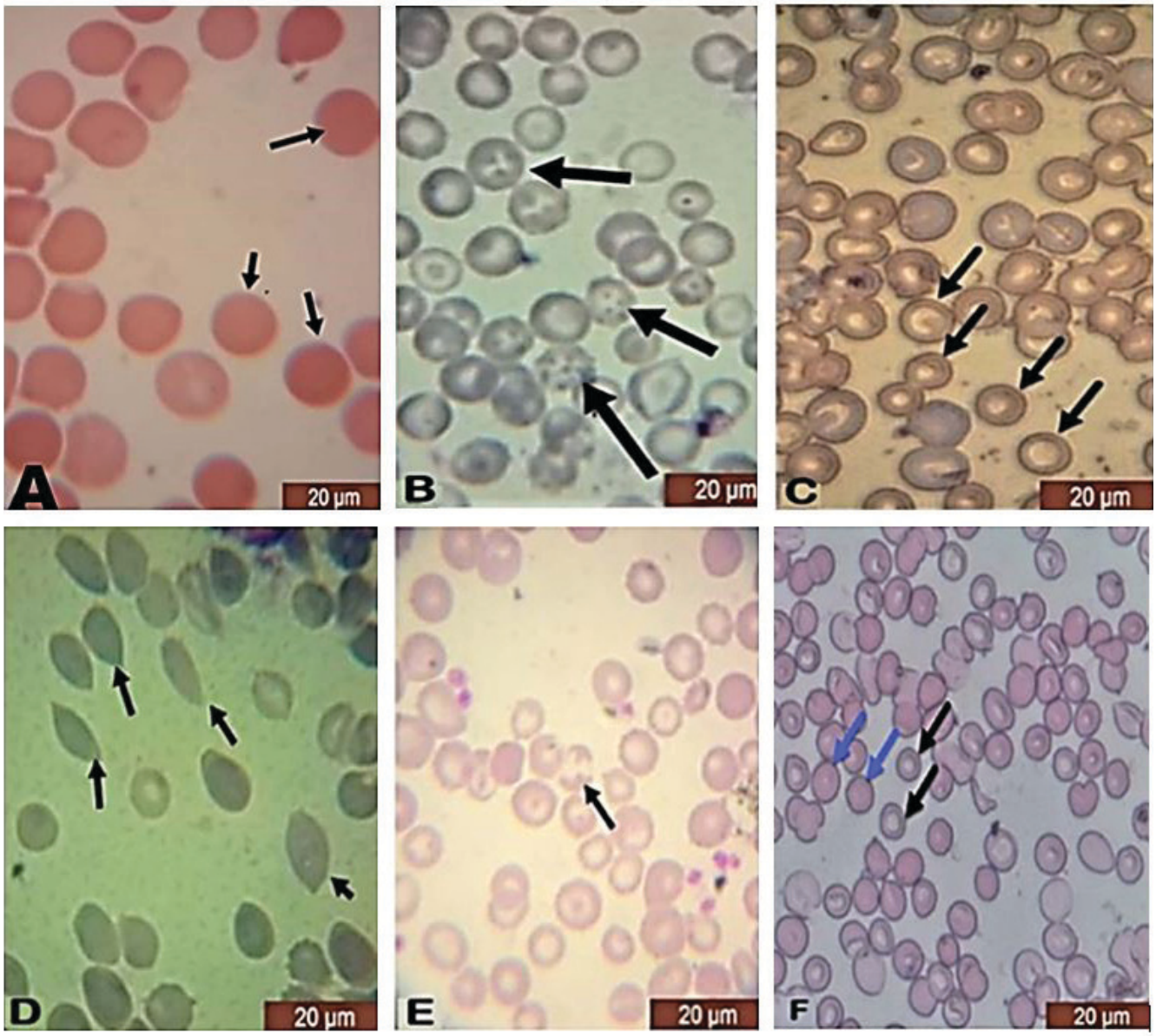

Figure 5: Blood smear of rats. (A) normal RBCs of control group, (B-E) RBCs of subgroup 3A (B) basophilic gran-ules in RBCs (C) hypo chromatic RBCs, (D) tear drops RBCs (E) hemolysis of RBCs. (F) RBCs of group B blue arrow nor-mal RBCs and black arrow hypo chromatic RBCs

The renal blood vessels, inter-tubular and glomerular blood capillaries, and perivascular edema mixed with erythrocytes were all congested in the kidneys retrieved from subgroup 3A (figure 3A). Thrombosis of the renal blood vessels with vacuolation of the glomerular endothelial cells, as well as necrosis of the glomerular tuft in some cases in association with focal area of lytic necrosis characterized by complete absence of renal tissue and replaced by eosinophilic debris with erythrocytes and few leukocytes (figure 3B) were also detected. In addition, extensive degenerative changes in the lining epithelium of the renal tubules were observed in the renal cortex, including vacuolation, hydropic degeneration, desquamation and necrosis with pyknotic nuclei in association with eosinophilic hyaline casts in the lumen of some renal tubules, mononuclear leukocytic cellular infiltrations in interstitial tissue with precipitation of lead pigment In most deteriorated epithelial cells, clumps of amorphous blue staining lead pigment were precipitated in varying quantities in the cytoplasm of the degenerated tubules of the renal medulla in conjunction with intra-nuclear eosinophilic inclusions (figure 3D). While in subgroup 3B showed improvement in the degenerative changes in the kidneys caused by lead acetate. A microscopical examination of

the kidney from subgroup 3B demonstrated a significant improvement in renal tissue histology when compared to subgroup 3A. There was mild congestion of the renal blood arteries and glomerular blood capillaries with normal histological structure of the glomeruli. Meanwhile, mild vacuolation of the endothelial cell lining of the glomerular tuft was observed in some cases (Figure 3E), along with mild degenerative changes in the lining epithelial cell of the renal tubules in the form of cloudy swelling, while control and pectin treated group's revealed normal renal histological structure.

Compared to the control group bone marrow (Figure 4a-b), a marked reduction in erythropoiesis was demonstrated in the bone marrow of lead intoxicated rats (subgroup 3A) as well as degeneration of myeloid cells, especially megakaryocytes, apoptosis of myeloid cells with the presence of intranuclear eosinophilic inclusion bodies was detected (Figure 4C-E). On the other side, a reduction in the pathological alterations induced by lead toxicity was observed in the bone marrow of rats in subgroup B as an increase in cell density affecting erythroid and myeloid cells with megakaryocytic hyperplasia in proportion to the other cells types (Figure 4F-G).

The Blood Smear (Field stain)

Blood smears were used to look for abnormal red blood cells as basophilic granulation, hypo chromatic, tear drops and hemolysis of RBCs Figure 5(B-E) which considered as important marker for lead toxicity, this may explain that lead causes anaemia and there were basophilic stippling cell noticed in subgroup 3A compared to other experimental groups.

Discussion

Lead is one of the most hazardous heavy metals on the human and animals. It is one of the most serious environmental pollutants. It used by humankind for many years due to its wide range of applications. Lead enters the body through a variety of routes, including the air, food, dust, soil, and water (35). Adult Wister rats were exposed to lead, had toxic effects in their blood, liver, and kidneys.

Oxidative stress is a primary mechanism of metal toxicity, and it was identified as a significant factor in our investigation when we discovered an altered redox state in treated rats' tissues as well as hematological problems (5). The activity of Aminolevulinic acid dehydratase (ALAD) is severely inhibited by lead, which disrupts haeme anabolism (36). The significant decrease of hematological indices (RBCs, Hb, MCH and MCHC) in lead acetate intoxicated rats indicated a state of anemia. This finding may be attributed to chelating properties of lead acetate (37). Lead can bind to essential minerals in the body, producing a variety of physiological problems in addition to affecting protein production and inhibiting hemoglobin formation (37). The protective effect of pectin as demonstrated in the current study was consistent with previous findings (18) which recorded the pectin's ability to chelate metals in the digestive system and inhibit absorption while aiding their removal in the faces (14). RBCs, Hb, PCV %, MCV, MCH, and MCHC were all lower in microcytic hypochromic anemia (39). In the current study, subgroup 3A showed that lead toxicity to rats induced microcytic hypochromic anemia, as it decreased RBCs, Hb, PCV%, MCV, MCH and MCHC, which in accordance with the results reported previously (40). Lead inhibits several enzymes that are important for haeme synthesis (41), and lead suppresses enzymatic activities such amionlevulinic acid dehydratase (ALAD), and ferochelatase, which are all important for haeme production, this suppression leads to a problem with iron metabolism (18). However, on the other hand, subgroup 3B showed an improvement in blood indices (RBCs, Hb, PCV%, MCV, MCH, and MCHC) when compared to subgroup 3A which in the same line of previous work (38) showed that date pectin extract was effective when taken orally for one month boosted RBC, Hb, MCV and MCH levels significantly ($P \leq 0.05$). previous work (42) demonstrated that low-esterified pectin quickly forms complexes with divalent metals, including ions of hazardous elements (mercury, lead, and cadmium), hence, reduced the cytotoxic effects of heavy metals. Histopathological examination of bone marrow in subgroup 3A (Figure 4c, e) explained that a marked reduction in erythropoiesis as well as degeneration of myeloid cells, especially megakaryocytes and apoptosis of myeloid cells were observed. While in subgroup 3B (Figure 4f, g)

hyperplasia of megakaryocytes and restoring of normal histological structure of bone marrow was observed and this explained the anemic picture improvement that appeared in blood indices of rats co-treated with lead acetate and pectin. Lead toxicity in subgroup 3A causing abnormal red blood cells which clear in blood smear (field stain) shown in figure 5 B1-B4) development of basophilic granules, hypo chromatic, tear drops and heamolyzied RBCs, which are characteristics of anemia caused by lead poisoning (43, 44), as previously noted (45). While in subgroup 3B, figure (5C) showed, only hypo chromatic RBCs due to protective effect of pectin. Our investigations (Figure 1) revealed significant ($P \leq 0.05$) decrease in hepcidin gene expression of subgroup 3A in comparison by control and subgroup 3B. This finding is parallel to previous work (9, 10) reported that Hepcidin gene expression is reduced following experimentally induced anemia and hypoxia. This could explains the increased iron release from reticuloendothelial cells and Hepcidin participation in anemic conditions is revealed by increased iron absorption in these settings and iron transfer from endosomes to the cytoplasm has also been found to be inhibited by lead. Subgroup 3B showed significant elevation in hepcidin expression gene ($P \leq 0.05$) which attributed to Pectin's ability that chelate metals in the digestive system and inhibit absorption while aiding their removal in the faces (14). In present study, subgroup 3A showed that, serum ferritin (iron store) and iron levels decreased significantly, in despite of increment of TIBC levels compared to the control. This result is in agreement with previous work (46) revealed that lead poisoning can cause anemia by interfering with haeme production, resulting in iron shortage. More recently (4), rats exposed to lead were found to have lower serum iron and transferrin saturation levels. The significant increase of TIBC could be related to higher production of transferrin by the liver in an attempt to make the most of the iron that is available (47). More over subgroup 3B showed an increasing of serum iron and ferritin and decreased TIBC as pectin rich in galacturonic acid (GalA) effectively chelate heavy metals (13, 48) suggesting that iron (Fe) bound to pectin is utilized by rats and enhancing the final Hb content. The current study demonstrated that liver functions in lead acetate intoxicate group had significantly higher AST and ALT activities than that of the control. These differences could be due to the toxic effect of lead acetate, which causes those enzymes to be released by increasing hepatocyte permeability or damaging the cell membrane of hepatocytes. In addition, lead toxicity produced an increase in cellular basal metabolic rate, irritability, and destructive alteration of liver cells (5, 49). As well as the creation of free radicals by lead caused harmful effect on hepatocytes, which reinforced by our histopathological picture showing in fig (5A-D) in subgroup 3A. This figure showed diffuse hydropic degeneration of hepatocytes in the hepatic parenchyma, multifocal regions of coagulative necrosis of hepatocytes with pyknotic nuclei and extensive diffuse regions of lytic necrosis with mild vacuolar degeneration of hepatocytes. The same picture showed mild amelioration of histpathological alternation in subgroup B (fig5 E), which showed mild vacuolar degeneration of hepatocytes, as majority of the hepatic parenchyma appeared to be improved may be due to treatment with Low-esterified pectin rapidly forms complexes with divalent metals as lead which are poisonous decreasing heavy metal cytotoxicity (42, 50). Previous study (38) found that ALAD activities were boosted by pectin treatment and decreased lipid peroxidation product in rat, as well as a significant increase in erythrocyte-SOD (Super oxide dismutase) and GSH activities, indicating that pectin protects body cells from oxidative radicals caused by lead. The significant increase of urea and creatinine lead acetate treated group compared to the control agrees with previous work (6) which recorded oral dose of lead acetate caused a significant rise in blood urea and serum creatinine. These results confirmed histopathologically (fig6 A-D), showed perivascular edema admixed with erythrocytes with necrosis of the glomerular tuft and thrombosis of the renal blood vessels with severe localized lytic necrosis, necrosis of the convoluted tubule lining epithelium, and moderate vacuolation of the glomerular tuft endothelial cell lining with cloudy swelling of the lining epithelium of some renal tubules. Slight improvement was observed in subgroup 3B as reflected on significant decrease of these parameters compared to that of subgroup 3A. These findings have been confirmed by hispathological picture (fig 6 E), that revealed improvement in renal tissue histology. This improvement was in the forms of mild vacuolation of the endothelial cell lining of the glomerular tuft

with mild degenerative changes in the lining epithelial cell of the renal tubules epithelium of some renal tubules, mild congestion of the renal blood vessels and glomerular blood capillaries with normal histological structure of the glomeruli. These findings were hand in hand with the improvement of functions the liver and kidney recorded earlier (50) with aresinic toxicity and co-treatment with pectin. This may be due to pectin hastened the elimination of arsenic in faces by lowering intestinal absorption, preventing buildup, and reducing arsenic toxicity. The exposure to lead acetate in subgroup 3A reduced the GSH activities and increased Nitric Oxide (NO) activity when compared to control. This result is comparable to that of a previous study of lead toxicity (38) caused oxidative stress, depletion of rapid antioxidants, higher production of reactive oxygen and nitrogen species and activation of lipid peroxidation. Thus, increasing oxidative stress induced a decrease in GSH levels, resulting in a decrease in glutathione concentration (3, 7). While in subgroup 3B, there were an improvement, as TAC and GSH were significantly elevated while Nitric Oxide activity was reduced. This finding may attributed to ROS scavenging activity of pectin, which is known to be reliant on pectin structural properties (51, 52). Present study showed that serum TNF-α and IL-6 in subgroup 3A increased significantly compared to the control group. Smilar results of (53) found that exposure to low lead level caused an increase of pro-inflammatory cytokines, such as TNF-α with a corresponding increase in other cytokines, such as IL-10, a T cell cross-regulatory factor, suggesting possible interference of lead in the immunophlogosis system. Lead has been found to enhance TNF- α production in vitro by human peripheral mononuclear cells (54). TNF-α is made primarily by activated macrophages and lymphocytes at the site of inflammation, and it plays a role in local and systemic inflammatory reactions with IL-6 and IL-1. It participates in local and systemic inflammatory reactions. Previous work (55) found that lead boosted total TNF- α cell expression in PBMC (+1ng/mL LPS). Pervious study (56) showed that blood levels of interleukin 6 (IL-6) and tumor necrosis factor (TNF- α) of 56 male workers chronically exposed to lead were significantly higher than that of the control group. In the current study, both TNF-α and IL-6 showed significant reduction in subgroup B compared to the control. This explained previously that commercially available Pectin had a pro-inflammatory effect in the spleen of BALB/c mice, up regulating cytokine release, including IL-17, IFN-, and (TNF- α), independent of Gal-3 inhibition (57). Moreover, pectin had a similar reducing effect on (TNF- α) and IL-10 secretion resulting in a higher survival rate in endotoxin-shocked mice (58). Citrus pectin has also been proven to inhibit the production of interleukin-6 (IL-6) and lowered the inflammatory cytokine gene expression (59).

Conclusion

Present study on lead toxicity on rats demonstrated a hazards effect on Hepcidin expression gene, serum iron profile, blood indices, and liver and kidney functions, oxidative and pro inflammatory effects. So to manage lead toxicity it is necessary to use a specific chelating agent and in the same time. Pectin may be regarded nutritional items that could be utilized to reduce lead intestine absorption, minimize lead buildup, and ameliorate lead poisoning because they are both selective and effective in interacting with lead. We concluded that daily pectin ingestion is recommended in highly polluted areas with lead.

Acknowledgement

This work was supported through the Annual Funding track by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project No. AN000478; GRANTI656).

References

1. Mañay N, Cousillas A, Alvarez CTH. Lead contamination in Uruguay: The “La Teja” neighborhood case. Rev Environ Contamtoxicol 2008; 195: 93–115.

2. Klauder D, Petering HG. Anemia of lead intoxication: a role of copper. J Nutr 1977; 107: 1779–85.

3. Rehman K, Fatima F, Waheed I. Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem 2018; 119: 157–84.

4. Moshtaghie M, Malekpouri P, Dinko MR, Moshtaghie AA. Changes in serum parameters as-

sociated with iron metabolism in male rat exposed to lead. J Physiol Biochem 2013; 69: 297–304.

5. Andjelkovic M, Buha Djordjevic A, Antonijevic E, et al. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Int J Environ Res Public Health 2019; 16: e274. doi: 10.3390/ijerph16020274

6. Elayat W, Bakheetf MS. Effects of chronic lead toxicity on liver and kidney functions. J Med Lab Sci 2010; 1: 29–36.

7. Zhushan F, Shuhua X. The effects of heavy metals on human metabolism. Toxicol Mech Methods 2020; 30: 167–76.

8. Qian ZM, Xiao DS, Wang Q, et al. Inhibitory mechanism of lead on transferrin-bound iron uptake by rabbit reticulocytes: a fractal analysis. Mol Cell Biochem 1997; 173: 89–94.

9. Theurl I, Aigner E, Theurl M, et al. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood 2009; 113: 5277–86.

10. Theurl I, Schroll A, Nairz M, et al. Pathways for the regulation of hepcidin expression in anemia of chronic disease and iron deficiency anemia in vivo. Haematologica 2011; 96(12): 1761–9.

11. Muckenthaler MU. Fine tuning of hepcidin expression by positive and negative regulators. Cell Metab 2008; 8: 1–3.

12. Drochner W, Kerler A, Zacharias B. Pectin in pig nutrition, a comparative review. J Anim Physiol Anim Nutr (Berl) 2004; 88: 367–80.

13. Vakkalanka DR, MS Oic, Chau HK, et al. Pectic oligosaccharide structure-function relationships: prebiotics, inhibitors of *Escherichia coli* O157:H7 adhesion and reduction of Shiga toxin cytotoxicity in HT29 cells. Food Chem 2017; 227: 245–54.

14. Eliaz I, Hotchkiss AT, Fishman ML, Rode D. The effect of modified citrus pectin on urinary excretion of toxic elements. Phytother Res 2006; 20: 859–4.

15. Paskins H, Tanaka Y, Skoryna S, Moore W, Stara J. The binding of lead by a pectic polyelectrolyte. Environ Res 1977; 14: 128–40.

16. Ostapenko VA, Teplyakov AI, Chegerova TI. Efficiency of apple pectin for prophylaxis of lead incorporation in workers. Med Truda I Promyshlennaya Ekol 2001; 5: 44–7.

17. Siu W, Ko E, Lee H, et al. A food supplement with lead chelating effect: a preliminary study. J Heavy Met Toxic Dis 2017; 2: 16. doi: 10.21767/2473-6457/1000016

18. Okediran BS, Biobaku KT, Olaifa1 FH, Atata AJ. Haematological and antioxidant enzyme response to lead toxicity in male wistar rats. Ceylon J Sci 2017; 46: 31–7.

19. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1975; 28: 56–63.

20. Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the berthelot reaction for the determination of ammonia. Anal Chem 1977; 49: 464–9.

21. Jaffé MUDN. Welchen pikrinsäure in normalem harn erzeugt und über eine neue reaction des kreatinins. Biol Chem 1986; 10: 391–400.

22. Ceriotti F, Ceriotti G. Improved direct specific determination of serum iron and total iron-binding capacity. Clin Chem 1980; 26: 327–31.

23. Linpisarn S, Kricka LJ, Kennedy JH, Whitehead TP. Sensitive sandwich enzyme immunoassay for serum ferritin on microtitre plates. Ann Clin Biochem 1981; 18: 48–53.

24. Fischl J, Cohen SA. Simple colorimetric method for the determination of total iron-binding-capacity of serum. Clin Chim Acta 1962; 7: 121–3.

25. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37(2) :112–9.

26. Moron MS, Depierre BM. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochem Biophys Acta 1979; 582: 67–72.

27. Montgomery H, Doymock J. Colorimetric determination of nitric oxide. Analyst 1961; 86: 414–6.

28. Chen W, Jin W, Cook M, Weiner LH, Wahl MS. Oral delivery of group a streptococcal cell walls augments circulating TGF-beta and suppresses streptococcal cell wall arthritis. J Immunol 1998; 161: 6297–304.

29. Hegazy AM, Fakhreldin AR, Nasr SN. Monitoring of carcinogenic environmental pollutants in women's breast milk. Biomed Pharmacol J 2020; 13(1): 119–25.

30. Gasparino E, Del Vesco AP, Voltolini DM, et al. The effect of heat stress on GHR, IGF-I, ANT, UCP and Coxiii mRNA expression in the liver and muscle of high and low feed efficiency female quail. Br Poult Sci 2014; 55: 466–73.

31. Ragan P, Turner T. Working to prevent lead poisoning in children: getting the lead out. JAAPA 2009; 22: 40–5.

32. Suvarna SK, Layton C, Bancroft JD. 2012. Bancroft's theory and practice of histological techniques. 7th ed. Oxford : Churchill Livingstone, 2012.

33. Luna GL. Manual of histological and special staining techniques of the Armed Forces Institute of Pathology. 3rd ed. New York : McGraw Hill, 1968: 9–34.

34. Hassanen E, Khalaf A, Zaki A, et al. Ameliorative effect of ZnO-NPs against bioaggregation and systemic toxicity of lead oxide in some organs of albino rats. Environ Sci Pollut Res 2021; 28(28): 37940-–52.

35. Herman D, Geraldine M, Venkatesh T. Evaluation, diagnosis, and treatment of lead poisoning in a patient with occupational lead exposure: aA case presentation. J Occup Med Toxicol 2007; 2: 7–10.

36. Dorward A, Yagminas A. Activity of erythrocyte δ-aminolevulinic acid dehydratase in the female cynomolgus monkey (*Macaca fascicularis*): kinetic analysis in control and lead-exposed animals. Comp Biochem Physiol Part B Comp Biochem 1994; 108: 241–52.

37. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 2006; 11: 114–27.

38. Nesrine S, Ouldali O, Bekara A, et al. Effect of pectin extract of date (*Phoenix dactylifera* l) on erythrocytes oxidative damage and hematological parameters induced by lead in males rats. J Appl Environ Biol Sci 2016; 6(10): 41–9.

39. Zhang X, Xie P, Li D, Shi Z. Hematological and plasma biochemical responses of crucian carp (*Carassius auratus*) to intraperitoneal injection of extracted microcystins with the possible mechanisms of anemia. Toxicon 2007; 49: 1150–7.

40. El-Bahr SM, Amal ME, Nashwa EG, et al. Biosynthesized iron oxide nanoparticles from *petroselinum crispum* leaf extract mitigate lead-acetate-induced anemia in male albino rats: hematological, biochemical and histopathological features. Toxics 2021; 9(6): e123 . doi: 10.3390/toxics9060123

41. Aly MH, Kim HC, Renner SW, et. al. Hemolytic anemia associated with lead poisoning from shotgun pellets and the response to succimer treatment. Am J Hematol 1993; 44: 280–3.

42. Dalia MEN. Effect of using pectin on lead toxicity. J Am Sci 2010; 6(12): 541–54.

43. Alleman H, Consendey B, Lob M. Lead poisoning by cutaneous drug absorption. Swiss Med Wkly 1986; 116: 888–91.

44. Ouldali O, Meddah M, Slimani M, et al. Beneficial effects of carrot pectin against lead intoxication in waster rats. Int J Green Pharmacy 2011; 5: 126-–31.

45. Noori M, Mohammad H, Zahra H, et al. Effects of chronic lead acetate intoxication on blood indices of male adult rat . DARU J Pharmaceut Sci 2003; 11(4): 147.

46. Rey V, ´Azquez G, Guerrero GA. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue Cell 2007; 39(3): 151–60.

47. Elshemy MAE. Iron oxide nanoparticles versus ferrous sulfate in treatment of iron deficiency anemia in rats. Egypt J Vet Sci 2018; 49: 103–9.

48. Tomihiro M, Akira N, Kiyoshi E. Iron bound to pectin is utilized by rats. Br J Nutr 2011; 106: 73–8.

49. Ibrahim NM, Eweis EA, El-Beltagi H S, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2012; 2: 41–6.

50. Fahmy HA, Sayed AEF, Omar TM. Effect of low and high-esterified pectin on arsenic toxification in rats. J Biol Chem Environ Sci 2008: 3: 11–33.

51. Liu SX, Shi L, Xu YY. Optimization of pectin extraction and antioxidant activities from Jerusalem artichoke. Chin J Oceanol Limnol 2016; 34: 372–81.

52. Wang W, Xiaobin M, Peng J. Characterization of pectin from grapefruit peel: a comparison of ultrasound–assisted and conventional heating extractions. Food Hydrocolloids 2016; 61: 730–9.

53. Rrocova Z, Macela A, Kroca M, Hernychova L. The immunomodulatory effect(s) of lead and cadmium on the cells of immune system in vitro. Toxicol In Vitro 2000; 14(1): 33–40.

54. Guo T, Mudzinski S, Lawrence D. The heavy metal lead modulates the expression of both TNF-alpha and TNF-alpha receptors in lipopolysaccharide-activated human peripheral blood mononuclear cells. J Leukoc Biol 1996; 59(6): 932–9.

55. Turner MD, Nedjai B, Hurst T. Pennington DJ. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta 2014; 1843(11): 2563–82.

56. Anna MG, Michal D, Aleksandra K, et al. The effect of subacute lead exposure on selected blood inflammatory biomarkers and angiogenetic factors. J Occup Health 2018; 60(5): 369–75.

57. Merheb R, Abdel-Massih RM, Karam MC. Immunomodulatory effect of natural and modified citrus pectin on cytokine levels in the spleen of balb/c mice. Int J Biol Macromol 2019; 121: 1–5. doi: 10.1016/j.ijbiomac.2018.09.189

58. Popov SV, Ovodov YS. Polypotency of the immunomodulatory effect of pectins. Biochemistry 2013; 78: 823–35.

59. Ishisono K, Yabe T, Kitaguchi K. Citrus pectin attenuates endotoxin shock via suppression of toll-like receptor signaling in peyer's patch myeloid cells. J Nutr Biochem 2017; 50: 38–45.

PEKTIN VPLIVA NA IZBOLJŠANJE HEMATOLOŠKIH IN BIOKEMIJSKIH PARAMETROV, HISTOPATOLOGIJE, BIOLOŠKIH OZNAČEVALCEV OKSIDATIVNEGA STRESA, CITOKINOV TER IZRAŽANJE GENA ZA HEPCIDIN PRI S SVINCEM POVZROČENI TOKSIČNOSTI PRI PODGANAH

S. M. El-Bahr, S. Al-Sultan, A. F. Hamouda, S. A. E. Atwa, S. Y. Abo-Kora, A. A. Amin, S. Shousha, S. Alhojaily, A. Alnehas, R. R. Elzogby

Izvleček: Objav o zaščitnem učinku pektina pred toksičnostjo svinca pri podganah ni na voljo. Da bi proučili ta učinek, smo 40 samcev podgan razdelili v 3 skupine. V prvi, kontrolni skupini je bilo 10 podgan. V drugi skupini je bilo 10 podgan, ki so v poskusnem obdobju (8 tednov) prejemale pektin v odmerku 100 mg/kg telesne teže. V tretji skupini je bilo 20 podgan, ki so 4 tedne dnevno prejemale svinčev acetat v odmerku 400 mg/kg telesne teže. Tretja skupina je bila nato razdeljena v dve podskupini (3A in 3B). V podskupini 3A je bilo 10 podgan, ki so še naprej 4 tedne prejemale svinčev acetat v enakem odmerku, v podskupini 3B pa je 10 podgan prejemalo svinčev acetat in pektin. Vzorci krvi so bili odvzeti po 2, 4 in 8 tednih od začetka poskusa. Ob koncu poskusa so bili odvzeti še jetra, ledvice in kostni mozeg. Svinčev acetat je povzročil anemijo šele po štirih tednih, kar se je kazalo v zmanjšanih vrednostih Hb, PCV, MCV, MCH in MCHC. V skupini podgan, ki so prejemale svinčev acetat, so te vrednosti do konca poskusa ostale nizke. Koncentracije serumskega feritina, železa, skupne antioksidativne kapacitete (TAC), reduciranega glutationa (GSH) in izražanje jetrnega gena za hepcidin so se pri podganah, ki so prejemale svinčev acetat, znatno zmanjšale v primerjavi s kontrolo. Aktivnosti ALT in AST ter koncentracije sečnine, kreatinina, dušikovega oksida (NO), TNF-α, IL-6, skupne kapacitete vezave železa (TIBC) in svinca so se v skupini, ki je prejemala svinčev acetat, znatno povečale v primerjavi s kontrolno skupino. Najvidnejše spremembe pri podganah, ki so prejemale svinec, so bile jetrna degeneracija in krvavitve, ledvična nekroza in apoptoza mieloidnih celic. Spremembe, povezane s svinčevim acetatom, so se izboljšale s sočasnim zdravljenjem s pektinom, vendar normalne kontrolne vrednosti niso bile dosežene. Zaključili smo, da je pektin priporočljiv za zaščito pred toksičnostjo svinčevega acetata pri podganah.

Ključne besede: svinčev acetat, toksičnost, pektin, hepcidin, biološki označevalci oksidativnega stresa, histopatologija