Effect of using pectin on lead toxicity

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ABSTRACT: Lead has many undesired effects on humans and animals, including neurological, behavioral, respiratory, visual, growth retardation, hematological immunological, renal, hepatic. **The aim of** the present study was to investigate the alterations in biochemical parameters in serum and blood due to lead retention in blood, organs and estimating the role of low and high esterified pectin in alleviating the negative effects of lead. **Material and Methods**: Sixty male *albino* rats which were divided into ten groups (6 rats for each). The first group (was fed on basal diet ;normal control). Groups 1,2 and 3 [which were fed on basal diet and administrated lead acetate (LA) daily once a time for 30 days by gavages at three different concentrations 61.94, 30.97 and 15.49 mg /Kg bw (1/4, 1/8, and 1/16 of lead acetate LD_{50};positive control]. Groups 4,5 and 6 [were fed on basal diet containing 10% low esterified pectin (LEP, DE 31%) and administered the same LA doses]. Groups 7,8 and 9 [were fed on basal diet containing 10% high esterified pectin (HEP, DE 73.5%) with the administration of the same LA doses]. **Results** obtained showed that LA significantly induced a decrease in body weight, serum total protein, albumin, globulin, total billirubin, direct billirubin, indirect billirubin, RBCs and WBCs counts, blood haemoglobin (Hb), heamatocrite values (PVC), serum triidothyronine (T3)and thyroxin (T4) levels. In the contrary, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AlP), gamma glutamyl transferase (γ-GT) activities, serum urea, uric acid and creatinine were significantly increased in positive control rat groups. Additionally, treatment of rats with LA led to a considerable increase in accumulation of the metal in the blood, liver, kidney, brain, heart and bones compared with the normal group. LEP and HLP significantly decreased the effect of LA on the tested parameters and level of lead in different organs. Histopathological examination clearly indicated that LEP or HEP eliminated from the harmful effect of LA on liver, kidney and brain tissues. **In conclusion**, LEP and HLP have beneficial effects which could be able to antagonize lead toxicity. Moreover, LEP was contributed to fast elimination of the lead acetate to blood, organs and bones, whereas HEP removed lesser amount of lead. It could be recommended that LEP has a good effect to bind material of lead and should be incorporated into human food to reduce the hazards toxicity of lead pollution of food and water.

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Key words: Lead toxicity, esterified pectin, Histopathological examination

1-INTRODUCTION

Lead is a pervasive and persistent environmental pollutant that can be detected in almost all phases of environment and biological systems. Lead dispersion in ambient air, in many foods, in drinking water, and in dust, Khotimchenko *et al,* (2007) and especially in the 20th century, because of the industrial applications, Landrigan *et al.*, (2000). Although lead is one of the most useful metals, it is also one of the most toxic, Shotyk and Le Roux (2005). It was indicated that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies, Hao *et al.*,(2002); Patrick, (2006), reproductive dysfunctions ,Marchlewicz *et al.*, (1993). Moreover, lead acetate and lead phosphate are listed as carcinogens.Inorganic lead compounds were classified as 2B chemicals by IARC, Pulido and Parrish, (2003). It was reported that lead increased the level of lipid per-oxidation,Upasani *et al.*, (2001). It can lead to inhibition of the activities of antioxidant enzymes, including glutathione peroxidase, catalase and superoxide dismutase, Silbergeld *et al.*, (2000). Furthermore, generation of reactive oxygen species (ROS), stimulation of lipid peroxidation and depletion of antioxidant reserves which was postulated to be major contributors to lead-exposure related diseases ,Patrick, (2006).

Pectin is the ionic plant polysaccharides widely used in food industry because of their gelling and thickening properties, Thakur *et al*, (1997). Pectin is a group of complex polysaccharides that contain 1,4-linked-d-galacturonic acid , Willats *et al*, (2001). There are three major pectic polysaccharides: homogalacturonans, rhamnogalacturonans-I, and rhamnogalacturonans-II. Natural pectins are highly esterified and contain more than 50% of esterified carboxyl groups, whereas LE pectins can be prepared Ridley *et al, (*2001). The number of esterified carboxyl groups determines the degree of

esterification, which is one of the important chemical parameters of pectins ,Schols and Voragen (1996).

It was shown that some physiological effects, for example, interactions with bile acids or drugs, may depend on the pectin structure, Dongowski *et al*, (1997).

The goal of this study was to determinate the relationship between structure of the pectin compounds with a high degree of esterification (HE pectin), and low esterified pectin (LE pectin) and their effects on lead absorption, retention, and removal in laboratory rats.

2-MATERIALS AND METHODS

2.1.Materials:

2.1.1.Chemicals and kits:

1. Pure lead acetate was purchased from Sigma Chemical (St. Louis, Mo). All other chemicals were of the highest available quality.

2. Genu® citrus pectin type 105 rapid set has high degree of estrification (DE) 73.5% and partially esterified Genu® citrus pectin type LM 104 As (31%DE) were obtained from Copenhagen pectin, Lille Skenved, Denmark.

3. Nitric acid 69% analar BDH and perchloric acid 70% Aldrich.

4. Biomerieux kits were obtained from Randox Laboratories Ltd., Diamond Road, Crumlin Co., Antrim, United Kingdom, BT294QY.

2.1.2. Experimental animals:

-Sixty male *albino* rats weighing 100 ± 5 g were obtained and housed in Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. The rats were kept under normal health laboratory conditions and fed on basal diet for one week. Water and basal diet were provided *ad libitum.* The basal diet consisted of protein (casein) 20%, cellulose 10 %, salt mixture 4%, vitamin mixture 1%, corn oil 10% and corn starch 55% according to (Pell *et al.* 1992). The adaptation period was one week.

-Rats were weighted twice weekly, total feed intake of each rat was weighted and feed conversion efficiency (gain weight of rat/ total feed intake, g) was calculated.

-Animals were weighted and randomly divided into 10 groups each one containing six rats, as follows:

Normal control: fed on basal diet.

Group 1: Positive control 1, fed on basal diet and administered 1/4 LD_{50} of LA.

Group 2: Positive control 2, fed on basal diet and administered 1/8 LD_{50} of LA.

Group 3: Positive control 3, fed on basal diet and administered 1/16 LD_{50} of LA.

Group 4: Fed on basal diet containing10% low esterified pectin (LEP) and administered 1/4 LD_{50} of LA.

Group 5: Fed on basal diet containing 10% low esterified pectin (LEP) and administered 1/8 LD_{50} of LA.

Group 6: Fed basal diet containing 10% low esterified pectin (LEP) and administered 1/16 LD_{50} of LA.

Group 7: Fed basal diet containing 10% high esterified pectin (HEP) and administered 1/4 LD_{50} of LA.

Group 8: Fed basal diet containing 10% high esterified pectin (HEP) and administered 1/8 LD_{50} of LA.

Group 9: Fed basal diet containing 10% high esterified pectin (HEP) and administered 1/16 LD_{50} of LA.

2.2. Methods:

2.2.1. Low and high esterified pectin:

2.2.1.1. Determination of galactouronic acid: The pectin content of low and high esterified pectins were determined as galactouronic acid by high performance liquid chromatography as described by Hicks *et al.,* (1985) with modification: 200 mg of low and high esterified pectin, dispersed in 1 ml of iced-cold 80% sulfuric acid, was allowed to set at 25 ºC for 18 hours. The sample was then diluted to 13 ml, sealed in a vail, and placed in a boiling water bath for 5 hours. The resulting dark solution was neutralized with solid calcium carbonate, filtered (0.2 µm) and injected into the chromatography.

Galactouronic acid was identified by a Hewlett Packard HP 1050 High performance liquid chromatography (HPLC) equipped with refractive index 1047 HP, Column compartment was set at 85 ºC, degaser and autosmpler. The chromatograph was fitted with Bio Rad HPX-87-C model (30cm× 7.8m id.) Isocratic elution system was used by deionized water at the flow rate 0.8ml/min.

2.2.2.1. Determination of degree of esterification: Methoxyl content was determined by the measurement of methanol liberated on saponification of the pectin according to the method described by Speirs *et al.,* (1980) as follows: 2 g of tested high and low pectins were homogenized with 150 ml water; the samples were saponified by the addition of 20 ml of 1M NaOH then allowed to stand for 30 min. at room temperature. The alkali was then neutralized by the addition of an equivalent amount of 0.5M HCl. The resulting acid mixture was transferred to 250ml volumetric flask and made up to the volume with distilled water. The contents of the flask were centrifuged at 12000 rpm for 20 min. the supernatant

was decanted off and retained until analysis of methanol released by using gas chromatography (Knuth, *et al.,* 1984 and litchman and Upton, 1972).

Gas chromatography condition: Analysis was performed on Hewlett- Packard Model 5890 gas chromatography equipped with flame ionization detector. The instrument was also equipped with HP-1 column (cross-lanked methyl silicone) 30 m x0.53 mmx 0.88 µm film thickness. Detector and injection port temperature was 250 ºC for each, nitrogen (15ml/min) was used as a carrier gas and hydrogen (15 ml/min and air 240ml/min was used for the flame operation. On-column injections of 1.0µl were used for all samples and standards. Columns were pre-conditioned with a nitrogen flow (15 ml/min) for 1 hr. at 30 ºC, then programmed at 4 ºC/min to 150 and held for 15 min. The reactive area under the peaks obtained from the chromatogram may be used to calculate the methoxyl content of the sample using the following relationship.

Methoxyl content (W/W)% = $1.211A_1 / (A_2 \times W)$. When: A_1 = area under the peak of sample. A_2 = area under the peak of standard. W = weight of sample (g).

Degree of esterification (DE) = (methoxyl content × 612%)/ galactouronic acid%.

2.2.2. Biochemical assay:

Blood samples were collected from the animals. Heparin was used as an anticoagulant. Serum was kept frozen at –20 ºC for biochemical assayas following:

-Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Bergmeyer and Harder (1986).

-Alkaline phosphatase (Al P; EC 3.1.3.1) activity was measured at 405 nm using the method of Varley *et al* (1980).

- Gamma -GT was measured according to the method described by Szasz (1960).

-Serum total protein (TP) was analyzed using the method of Lowry *et al* (1951).

- Albumin concentrations were determined by the method of Doumas *et al* (1977)

- Globulin concentrations were determined by difference (TP - albumin).

-Serum total billirubin and direct billirubin was measured using the method of Walters and Gerade (1970). Indirect billirubin was calculated by difference between Serum total billirubin and direct billirubin. - Creatinine, urea and uric acid were determined by using the methods described by Larsen (1972), Patton and Crouch (1977) and Caraway (1955).

-Total Triiodothyronine (T3) and Total Thyroxin (T4) hormones were estimated in serum samples using DIMA GmbH Diagnostics kits, Goettingen, Germany according to the method described by Young *et al,* (1975) and Sterling (1975), respectively

2.2.2. Hematological evaluation:

Leucoytes count (WBC_s), red blood cell (RBC_s), blood haemoglobin (Hg) and haematocrite value (PVC) estimated by Wintrobe (1967); Dacie and Lewis (1975); Leong *et al* (2003) and Burch and Siegel (1971),respectively.

2.2.3. Measurement of lead concentration:

2.2.3.1. In the blood;

Approximately 1 ml of blood was digested with nitric and perchloric acid mixture (Kolmer *et al.*, 1951). Two to three blank samples were run simultaneously with each batch of the digestion where bio-sample was substituted by de-ionized distilled water. The equal quantity of acid mixture was added to the blank, standard and test digestion tubes during low heat digestion.

2.2.3.2. In organs and feces;

At the end of the experimental period, organs (liver, kidney, brain, heart and bones) were isolated and feces were collected and stored at -20ºC for wet digestion and analysis of lead content.

To accurately weight of tissue and feces in 125 ml Erlenmeyer flask, add glass beads and 25ml deionized water, add 10 ml 1:2 (equal volumes) mixture of concentrated HNO_3 and $HClO_4$. Boil the sample until the solution is clear ,Parker *et al*, (1967). Transfer the solution quantitatively to 100 ml volumetric flask. Dilute to volume with deionized water and mix well.

The lead concentrate in blood, organs and feces was estimated using a Perkin Elmer Plasma 400, Emission spectrometer, Koirtyohann (1994). The average reading of the blanks was first subtracted from that of standard and test samples and then calculation was made for their lead concentration in µg/dl of blood and µg/g of wet tissue and feces.

2.2.4. Histopathological studies:

Tissue specimens from rat's liver, kidney and brain were fixed in 10 % neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hemato-xylene and eosin according to Bancroft *et al* (1996). The degree of injury was estimated using an ordinal scale modified from Palaa and Charbonneau (1994).

2.2.5. Statistical analysis:

Each parameter was analyzed separately by using one-way analysis of variance (ANOVA). For determining differences between groups, the Duncan test was used. All *p* values of <0.05were considered to be significant.

3. RESULTS

As a majority of polysaccharides, pectins are heterogeneous compounds regarding structure, molecular weight, and physicochemical properties. These parameters of pectins vary from one fruit species to another and also during the different developmental stages of the fruit (Chang *et al*, 1994) as well as in the process of chemical and enzymatic modifications.

The galactouronic acid concentration in high and low esterified pectin were 75.72 and 78.53%, respectively. The assay showed degree of esterification to be approximately 73.5 and 31% respectively in high and low esterified pectin respectively**.**

3.1. Growth of rats:

Data in Table (1) show changes in the weight and the feed conversion efficiency (FCE) of the tested rat groups. Significant differences between groups in weight gain were noticed. The highest accumulated weight gain was recorded by G9 and normal control which fed 10% HEP + 1/16 LD_{50} of Pb and normal control, respectively. However, the lowest accumulated weight gain was observed in positive control rat groups (G1, G2 and G3). No significant difference appeared for feed intake of all tested rat groups. At the same time, feed conversion efficiency (FCE) was also decreased by administered oral doses of lead acetate without fed HEP or LEP (G1, G2 and G3).

Data presented in Table (3), show significant differences among tested rat groups in their serum total protein, albumin, globulin, total billirubin, direct billirubin and indirect billirubin at the end of experimental period. Positive rat groups (G1, G2 and G3) had significant decrease compared with treated rat groups and normal control. There were no significant differences observed between the tested rat groups in A/G ratio.

3.2. Biochemical assay:

3.2.1. 1.Liver function

The level of serum ALT, AST, ALP and γ-GT activities are presented in Table (2). Significant increases were observed in these enzymes activity in rat groups administered lead acetate doses without fed HEP or LEP. There was friary difference between normal control, G6 (fed 10% LEP + 1/16 LD_{50} of Pb) and G9 (fed 10% HEP + 1/16 LD_{50} of Pb) at the end of experimental period in serum ALT and AST activities. Moreover, No significant difference was observed in serum ALP and γ- GT activities among tested rat groups expect positive control rat groups (G1, G2 and G3) at the end of experimental period.

Table (1): Weight gain, feed intake and feed efficiency ratio of rats fed on 10 % LEP or HEP and administrated different doses of lead acetate

Rat groups	Initial weight (g.)	Final weight (g.)	Weight gain (g.)	Feed intake (g.)	Feed efficiency ratio
Normal control	100.23 ± 6.91^{a}	204.16 ±	103.92 ±	375.76 ±	0.280 ±
G1 (1/4 LD_{50} of Pb)	101.51 ±	170.83 ± 5.18^{b}	69.32 ± 3.72^{d}	330.23 ±	0.210 ±
G2 (1/8 LD_{50} of Pb)	99.72 ±	169.71 ± 3.46^{b}	70.67 ± 3.89^{d}	316.19 ±	0.223 ±
G3 (1/16 LD_{50} of Pb)	99.23 ±	173.45 ± 6.27^{b}	74.22 ± 2.98^{d}	323.49 ±	0.230 ± 0.010 ^{cd}
G4 (fed 10% LEP +1/4 LD_{50} of Pb)	100.58 ±	191.02 ± 16.09^{ab}	90.44 ± 7.01^{bc}	366.50 ±	0.247 ± 0.006 ^{cd}
G5 (fed 10% LEP +1/8 LD_{50} of Pb)	101.84 ±	203.20 ±	101.53 ± 3.48 ^{ab}	381.03 ±	0.267 ± 0.006 ^{ab}
G6 (fed 10% LEP +1/16 LD_{50} of Pb)	100.49 ±	202.49 ±	101.91 ± 6.79 ^{ab}	378.20 ±	0.270 ± 0.010 ^{ab}
G7 (fed 10% HEP +1/4 LD_{50} of Pb)	100.49 ±	188.45 ± 1.74^{ab}	88.05 ± 6.74^{c}	345.24 ±	0.240 ± 0.010 ^{de}
G8 (fed 10% HEP +1/8 LD_{50} of Pb)	100.71 ±	196.39 ±	95.68 ± 5.52^{bc}	372.97 ±	0.257 ± 0.006 ^{bc}
G9 (fed 10% HEP +1/16 LD_{50} of Pb)	100.86 ±	206.26 ±	115.53 ±	400.74 ±	0.263 ± 0.005 ^{ab}
L.S.D.	11.6405	15.6354	9.2249	10.0508	0.0132

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test.

Table (2): Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) andgamma glutamyl transferase (Gamma GT) of rats fed on 10% LEP or HEP and administrated different doses of lead acetate

Rat groups	ALT		AST		ALP		Gamma GT	
	Zero time	End	Zero time	End	Zero time	nd	Zero time	End
Normal control	39.20 ± 1.24 ^{af}	41.26 ± 2.31^{a}	82.34 ± 3.77^{a}	84.66 ± 4.35^{a}	144.33 ± 5.75^{a}	152.34 ± 8.47^{a}	4.62 ±	4.30 ±
G1 (1/4 LD_{50} of Pb)	39.01 ± 2.83 ^{af}	61.65 ±	80.25 ± 5.09^{a}	128.97 ±	152.80 ± 9.50^{a}	263.02 ±	4.41 ±	6.14 ± 0.81^{b}
G2 (1/8 LD_{50} of Pb)	37.63 ±	62.12 ±	80.84 ± 2.60^{a}	128.83 ±	145.02 ± 8.06^{a}	257.75 ±	4.23 ±	5.70 ± 0.28^{b}
G3 (1/16 LD_{50} of Pb)	37.93 ± 1.56 ^{af}	58.73 ±	79.74 ± 2.83^{a}	124.10 ±	143.86 ± 8.04^{a}	217.93 ± 8.31 ^{b}	4.67 ±	5.73 ± 0.23^{b}
G4 (fed 10% LEP+ 1/4 LD_{50} of Pb)	37.77 ± 1.14 ^{af}	48.48 ± 4.33 ^{bc}	81.14 ± 2.74^{a}	94.00 ± 5.09^{b}	151.16 ± 4.89^{a}	149.78 ± 6.85 ^{c}	4.53 ±	4.46 ±
G5 (fed 10% LEP+ 1/8 LD_{50} of Pb)	39.82 ± 1.70 ^{af}	43.26 ± 2.19 ^{bc}	80.83 ± 3.31^{a}	93.32 ± 2.89^{b}	150.74 ± 9.30^{a}	152.10 ± 10.29 ^{c}	4.29 ±	4.51 ±
G6 (fed 10% LEP+ 1/16 LD_{50} of Pb)	39.67 ± 2.39 ^{af}	40.07 ± 1.66 ^{af}	80.09 ± 4.21^{a}	80.61 ± 5.76^{c}	154.66 ± 7.10^{a}	144.30 ± 11.09 ^{c}	4.60 ±	4.04 ±
G7 (fed 10% HEP+ 1/4 LD_{50} of Pb)	37.16 ±	52.16 ± 3.57^{b}	79.74 ± 3.11^{a}	100.10 ± 6.46^{b}	149.43 ± 9.68^{a}	158.50 ± 7.29 ^{c}	4.35 ±	4.38 ±
G8 (fed 10% HEP+ 1/8 LD_{50} of Pb)	38.28 ±	44.28 ± 1.34 ^{cd}	80.37 ± 3.93^{a}	94.89 ± 4.27^{b}	151.37 ± 10.22^{a}	149.92 ± 4.10 ^{c}	4.11 ±	4.27 ±
G9 (fed 10% HEP+ 1/16 LD_{50} of Pb)	39.43 ±	40.53 ± 2.44 ^{af}	79.79 ± 4.30^{a}	85.03 ± 3.20^{c}	151.84 ± 6.49^{a}	143.43 ± 3.78 ^{c}	4.22 ±	4.40 ±
L.S.D.	4.6228		8.1085		13.1888		0.9308	

Each value represents the mean ± SE. The mean values with different superscript alphabets
indicate significant differences ($P \leq 0.05$) using LSD test.

3.2.2. **Kidney function:**

The level of serum urea, uric acid and creatinine are showed in Table (4). There was slight significant difference among the rat groups at zero time and rat groups fed 10 % LEP or HEP and administered different doses of lead acetate at the end of experimental period.

Table (3): Serum total protein (TP), albumin, globulin, A/G ratio, total, direct and indirect bilirubin of rats fed on 10 % LEP or HEP and administrated different doses of lead acetate

Rat groups	Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A/G ratio		Total bilirubin (mg/dl)		Direct bilirubin (mg/dl)		Indirect bilirubin (mg/dl)	
	Zero time	End	Zero time	End	Zero time	End	Zero time	End	Zero time	End	Zero time	End	Zero time	End
Normal control	6.76 ± 0.39^{bc}	5.77 ± 0.29^{cd}	4.2 ±	4.02 ±	2.74 ±	2.35 ±	1.45 ± 0.1^{ab}	1.49 ± 0.29^{ab}	0.231 ± 0.011^{bc}	0.253 ± 0.006^{b}	0.162 ± 0.01^{cd}	0.167 ± 0.008^{bc}	0.070 ±	0.086 ±
G1 (1/4 LD_{50} of Pb)	6.96 ±	4.48 ± 0.58^{g}	4.23 ±	2.61 ± 0.19^{g}	2.95 ±	1.87 ± 0.23^{g}	1.56 ± 0.06^{ab}	1.4 ± 0.15^{ab}	0.245 ± 0.013^{a}	0.515 ±	0.147 ± 0.002^{ab}	0.288 ±	0.098 ± 0.007^{ab}	0.201 ±
G2 (1/8 LD_{50} of Pb)	7.03 ±	4.92 ± 0.18^{ef}	4.34 ±	2.95 ± 0.14^{fg}	2.86 ±	1.87 ± 0.51^{g}	1.49 ± 0.25^{ab}	1.79 ± 0.35^{ab}	0.234 ± 0.003^{a}	0.485 ± 0.006^{ab}	0.144 ± 0.01^{ab}	0.279 ± 0.019^{cd}	0.089 ± 0.006^{ab}	0.2 ±
G3 (1/16 LD_{50} of Pb)	6.98 ±	5.48 ± 0.49^{de}	4.18 ±	3.04 ± 0.21^{ef}	2.89 ±	2.44 ± 0.26^{fg}	1.45 ± 0.05^{ab}	1.36 ± 0.27^{ab}	0.254 ± 0.013^{a}	0.495 ± 0.013^{ab}	0.152 ± 0.006^{ab}	0.252 ± 0.008^{b}	0.078 ± 0.006^{cd}	0.2 ±
G4 (fed10% LEP+1/4 LD_{50} of Pb)	6.97 ±	5.68 ± 0.58^{cd}	4.34 ±	3.34 ± 0.19^{cd}	2.95 ±	2.22 ± 0.38^{gh}	1.56 ± 0.06^{ab}	1.65 ± 0.51^{ab}	0.236 ± 0.004^{a}	0.264 ± 0.025^{cd}	0.152 ± 0.006^{ab}	0.162 ± 0.007^{de}	0.083 ± 0.002^{ab}	0.101 ± 0.01^{ab}
G5 (fed10% LEP+1/8 LD_{50} of Pb)	6.97 ±	5.03 ± 0.16^{ef}	4.26 ±	3.05 ± 0.18^{ef}	2.94 ±	2.29 ± 0.54^{fg}	1.45 ± 0.25^{ab}	1.51 ± 0.23^{ab}	0.236 ± 0.02^{a}	0.255 ± 0.008^{cd}	0.135 ± 0.004^{ab}	0.153 ± 0.009^{de}	0.083 ± 0.002^{ab}	0.101 ± 0.01^{ab}
G6 (fed10% LEP+1/16 LD_{50} of Pb)	6.95 ±	5.97 ±	4.28 ±	4.04 ±	2.95 ±	2.52 ±	1.37 ± 0.04^{ab}	1.41 ± 0.15^{ab}	0.233 ± 0.01^{a}	0.245 ± 0.013^{ab}	0.147 ± 0.001^{ab}	0.161 ± 0.003^{de}	0.084 ± 0.01^{ab}	0.104 ± 0.01^{ab}
G7 (fed10% HEP+1/4 LD_{50} of Pb)	6.99 ±	5.52 ± 0.58^{de}	4.19 ±	3.45 ± 0.1^{de}	2.79 ±	2.15 ± 0.29^{gh}	1.47 ± 0.17^{ab}	1.72 ±	0.229 ± 0.01^{a}	0.267 ± 0.004^{cd}	0.154 ± 0.006^{ab}	0.169 ± 0.01^{de}	0.077 ± 0.002^{ab}	0.1 ± 0.02^{ab}
G8 (fed10% HEP+1/8 LD_{50} of Pb)	7.04 ±	5.72 ± 0.34^{cd}	4.31 ±	3.45 ± 0.2^{de}	2.89 ±	2.64 ±	1.42 ± 0.25^{ab}	1.48 ± 0.29^{ab}	0.231 ± 0.01^{a}	0.267 ± 0.006^{cd}	0.135 ± 0.01^{ab}	0.153 ± 0.011^{de}	0.078 ± 0.002^{cd}	0.114 ± 0.01^{ab}
G9 (fed10% HEP+1/16 LD_{50} of Pb)	6.91 ±	5.52 ± 0.58^{de}	4.21 ±	3.36 ± 0.21^{de}	2.89 ±	2.61 ±	1.46 ± 0.17^{ab}	1.50 ± 0.14^{ab}	0.228 ± 0.01^{a}	0.247 ± 0.013^{ab}	0.152 ± 0.006^{ab}	0.144 ± 0.012^{de}	0.076 ± 0.002^{ab}	0.104 ± 0.01^{ab}
L.S.D.	0.5562		0.5255		0.6753		0.3284		0.0402		0.0179		0.01934	

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test.

3.2.3. **Level of thyroid hormones, triidothyronine (T3) and thyroxin (T4):**

The effect of feeding on LEP or HEP on serum T3 and T4 levels of rats administrated different doses of lead acetate were studied and the results are shown in Table (6). Normal control rats group had significantly the highest serum T3 and T4 levels. Meanwhile, group 1 (administered 1/4 LD_{50} of Pb) had significantly the lowest serum T3 and T4 levels.

3.3. Hematological evaluation:

Hematological parameters observed in the normal control and experimental rat groups are shown in Table (5).The RBCs and WBCs counts, blood haemoglobin (Hb) and haematocrite values (PVC) significantly decreased in positive control rat groups compared to normal control group at the end of experimental period. However, these parameters significantly increased in rat groups fed on 10% LEP or HEP. It could be noticed that, RBCs and WBCs counts in rat groups fed with LEP were higher than others being fed with HLP in the same concentration of lead acetate. There was a fairly significant difference among all treated rat groups and normal control rats group in Hb. Meanwhile, there was no significant differences in heamatocrite values (PVC) between all treated rat groups and normal control rats group expect G7 (fed on 10 % HEP and administrated 1/4 LD_{50} of lead.

Table (4): Serum urea, uric acid and creatinene of rats fed on10 % LEP or HEP and administrated different doses of lead acetate

Rat groups	Urea (mg/dl)		Uric acid (mg/dl)		Creatinine (mg/dl)	
	Zero time	End	Zero time	End	Zero time	End
Normal control	22.48 ± 1.37^{b}	21.34 ± 1.45^{b}	1.35 ± 0.11^{b}	1.39 ± 0.11^{b}	1.55 ± 0.04^{a}	1.59 ± 0.08^{a}
G1 (1/4 LD_{50} of Pb)	22.37 ± 0.79^{b}	44.98 ±	1.34 ± 0.14^{b}	2.11 ±	1.59 ± 0.08^{a}	2.69 ±
G2 (1/8 LD_{50} of Pb)	21.22 ± 1.49^{b}	44.20 ±	1.29 ± 0.19^{b}	2.06 ±	1.54 ± 0.06^{a}	2.49 ± 0.23^{b}
G3 (1/16 LD_{50} of Pb)	21.06 ± 1.46^{b}	40.93 ±	1.43 ± 0.10^{b}	2.03 ±	1.55 ± 0.05^{a}	2.26 ± 0.13^{c}
G4 (fed10% LEP+1/4 LD_{50} of Pb)	20.40 ± 3.31^{bc}	23.42 ± 1.52^{b}	1.41 ± 0.09^{b}	1.34 ± 0.09^{b}	1.59 ± 0.06^{a}	1.75 ± 0.09^{d}
G5 (fed10% LEP+1/8 LD_{50} of Pb)	21.95 ± 0.29^{b}	22.25 ± 1.78^{b}	1.38 ± 0.09^{b}	1.32 ± 0.15^{b}	1.57 ± 0.06^{a}	1.59 ± 0.10^{a}
G6 (fed10% LEP+1/16 LD_{50} of Pb)	20.59 ± 1.72^{b}	21.56 ± 0.87^{b}	1.44 ± 0.06^{b}	1.41 ± 0.08^{b}	1.57 ± 0.04^{a}	1.68 ± 0.04^{d}
G7 (fed10% HEP+1/4 LD_{50} of Pb)	21.28 ± 1.38^{b}	23.49 ± 1.70^{b}	1.45 ± 0.07^{b}	1.39 ± 0.22^{b}	1.63 ± 0.07^{a}	1.86 ± 0.06^{d}
G8 (fed10% HEP+1/8 LD_{50} of Pb)	20.24 ± 1.19^{bc}	22.59 ± 1.00^{b}	1.44 ± 0.14^{b}	1.42 ± 0.03^{b}	1.52 ± 0.05^{ef}	1.62 ± 0.02^{a}
G9 (fed10% HEP+1/416 LD_{50} of Pb)	21.47 ± 1.23^{c}	20.96 ± 1.60^{b}	1.37 ± 0.08^{b}	1.41 ± 0.14^{b}	1.50 ± 0.02^{ef}	1.66 ± 0.01^{de}
L.S.D.	5.0473		0.2762		0.1671	

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test.

Table (6): Serum Total Triiodothyronine (T3) and Total Thyroxin(T4) hormones of rats fed on 10 % LEP or HEP and administrated different doses of lead acetate

Rat groups	T3 (ng/ml)		T4 (nmol/L)	
	Zero time	End	Zero time	End
Normal control	1.74 ± 0.19^{cd}	2.56 ±	42.68 ± 1.84^{bc}	57.84 ±
G1 (1/4 LD_{50} of Pb)	1.78 ± 0.15^{cd}	0.90 ±	42.23 ± 3.87^{bc}	19.39 ± 2.15^{h}
G2 (1/8 LD_{50} of Pb)	1.87 ± 0.16^{cd}	1.15 ±	42.07 ± 2.91^{bc}	25.21 ±
G3 (1/16 LD_{50} of Pb)	1.81 ± 0.11^{cd}	1.55 ± 0.22^{e}	41.33 ± 3.38^{bc}	29.74 ± 1.91^{fg}
G4 (fed 10% LEP+ 1/4 LD_{50} of Pb)	1.94 ± 0.12^{bc}	1.83 ± 0.02^{cd}	40.98 ± 3.41^{cd}	35.21 ± 0.98^{ef}
G5 (fed 10% LEP+ 1/8 LD_{50} of Pb)	1.95 ± 0.26^{bc}	2.17 ± 0.11^{b}	42.24 ± 2.24^{bc}	46.00 ± 1.20^{b}
G6 (fed 10% LEP+ 1/16 LD_{50} of Pb)	1.84 ± 0.06^{cd}	2.42 ±	41.98 ± 2.91^{bc}	53.96 ±
G7 (fed 10% HEP+ 1/4 LD_{50} of Pb)	1.83 ± 0.07^{cd}	1.72 ± 0.08^{de}	40.32 ± 3.75^{cd}	34.21 ± 2.36^{ef}
G8 (fed 10% HEP+ 1/8 LD_{50} of Pb)	1.85 ± 0.10^{cd}	1.95 ± 0.07^{bc}	40.38 ± 4.30^{cd}	36.46 ± 2.37^{de}
G9 (fed 10% HEP+ 1/16 LD_{50} of Pb)	1.93 ± 0.09^{bc}	2.01 ± 0.23^{bc}	41.83 ± 1.17^{bc}	42.99 ± 2.94^{b}
L.S.D.	0.2354		5.3577	

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test.

Table (5): Blood leukocytes count (WBC_s), red blood cell (RBC_s), blood haemoglobin (Hg) and heamatocrite value (PVC) of rats fed on 10 % LEP or HEP and administrated different doses of lead acetate

Rat groups	WBC_s (N x 10^3/ul)		RBC_s (N x 10^6/ul)		Hg (g/dl)		PVC %	
	Zero time	End	Zero time	End	Zero time	End	Zero time	End
Normal control	14.33 ± 0.81 ^{bc}	14.81 ±	9.69 ± 0.47 ^{cd}	11.02 ±	13.99 ± 0.94 ^{bc}	14.68 ± 0.95 ^{bc}	49.47 ±	51.07 ±
G1 (1/4 LD_{50} of Pb)	14.86 ±	7.18 ± 0.42 ^{h}	9.62 ± 0.67 ^{cd}	7.18 ±	14.23 ± 0.28 ^{bc}	9.72 ± 0.56 ^{e}	49.89 ±	37.01 ± 1.98 ^{e}
G2 (1/8 LD_{50} of Pb)	14.72 ± 0.89 ^{ab}	8.52 ±	9.46 ± 0.55 ^{cd}	7.57 ± 0.49 ^{fg}	14.52 ± 0.51 ^{bc}	10.82 ± 0.51 ^{d}	49.34 ±	36.46 ± 3.53 ^{e}
G3 (1/16 LD_{50} of Pb)	13.93 ± 1.44 ^{bc}	8.93 ±	10.25 ± 0.77 ^{ab}	8.34 ± 0.64 ^{ef}	14.28 ± 0.55 ^{bc}	11.02 ± 0.60 ^{d}	49.46 ±	41.48 ± 5.71 ^{c}
G4 (fed 10% LEP + 1/4 LD_{50} of Pb)	14.34 ± 0.40 ^{bc}	11.76 ± 0.71 ^{ef}	9.85 ± 0.29 ^{cd}	10.11 ± 0.35 ^{ab}	14.58 ± 0.23 ^{bc}	14.60 ± 0.46 ^{bc}	48.17 ±	48.31 ±
G5 (fed 10% LEP + 1/8 LD_{50} of Pb)	14.15 ± 0.47 ^{bc}	12.59 ± 0.57 ^{de}	9.78 ± 0.48 ^{cd}	10.20 ± 0.38 ^{ab}	14.30 ± 0.54 ^{bc}	14.84 ± 0.29 ^{bc}	47.80 ±	47.98 ±
G6 (fed 10% LEP + 1/16 LD_{50} of Pb)	14.17 ± 0.21 ^{bc}	14.15 ± 1.03 ^{bc}	9.57 ± 0.34 ^{cd}	10.62 ± 0.34 ^{ab}	14.42 ± 0.45 ^{bc}	15.35 ±	48.59 ±	48.61 ±
G7 (fed 10% HEP + 1/4 LD_{50} of Pb)	14.27 ± 0.53 ^{bc}	10.67 ±	9.89 ± 0.81 ^{cd}	8.91 ± 0.40 ^{de}	14.23 ± 0.52 ^{bc}	13.71 ± 0.44 ^{c}	48.05 ±	45.23 ± 2.47 ^{b}
G8 (fed 10% HEP + 1/8 LD_{50} of Pb)	13.23 ± 0.65 ^{bc}	11.54 ± 0.57 ^{ef}	9.77 ± 0.34 ^{cd}	9.91 ± 0.24 ^{cd}	14.89 ± 0.68 ^{bc}	14.41 ± 0.79 ^{bc}	49.16 ±	47.93 ±
G9 (fed 10% HEP + 1/16 LD_{50} of Pb)	12.97 ± 0.34 ^{cd}	12.61 ± 0.24 ^{de}	9.95 ± 0.82 ^{cd}	9.89 ± 0.58 ^{cd}	14.49 ± 0.37 ^{bc}	15.19 ± 0.47 ^{ab}	49.30 ±	49.08 ±
L.S.D.	1.2892		0.9435		1.0976		3.8799	

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test.

3.4.Concentration of lead retention in blood, organs and feces:

The blood lead level of 0.35 lg/ml is the critical level of poisoning, but the deaths commence at 1.0 lg/ml (Radostits *et al*, 2000). Sometimes, blood lead level above this critical level may not be manifested by characteristic clinical signs in animals (Koh and Babidge, 1986), particularly in chronically exposed cases. Moreover, uptake by bone mineral is a function of the metal's plasma concentration, its affinity for the bone mineral, and its effect on the extracellular matrix. It is also a function of the degree of mineralization of the skeleton.

Treating the rats with Pb in the form of acetate led to a considerable increase of accumulation of the metal in the blood, liver, kidney, brain, heart and bones compared with the normal group. Data in Table (7) showed that significant increase was observed in all rat groups administered lead acetate. At rat groups fed on LEP or HEP, there was decrease in lead concentration of blood, organs and bones. It could be noticed that, the efficiency of LEP on removal lead from tested blood and organs were higher than HEP in the presence of the same doses of lead acetate. Also, there was no significant difference in the lead concentration of heart compared to normal control group and G6 (fed on 10 % LEP and administrated 1/16 LD_{50} of lead. In all groups of animals fed on LEP or HEP, there was significant increase lead concentration in feces compared to the positive control rat groups (G1, G2 and G3) indicating continuous lead elimination through the digestive tract. LEP was helped to increase the amount of lead being excreted with feces. Amount of lead in feces of rats fed on HEP was significant decrease from that of rats fed on LEP in different doses of lead.

In this study, the use of the pectin substances in animals preliminary exposed to high doses of lead acetate contributed to fast elimination of the metal from the organs, in particular, the bones. Low esterified pectin prevented lead absorption in the intestine, resulting in slowed tissue retention of lead, whereas HE pectin removed lesser amount of lead. These results were confirmed by enhanced concentration on lead in feces of rats treated with LEP and HEP.

Table (7): Lead content of blood, liver, kidney, brain, heart, bone and feces of rats fed on 10 % LEP or HEP and administrated different doses of lead acetate

Rat groups	Blood (µg/dl)	Liver (µg/g)	Kidney (µg/g)	Brain (µg/g)	Heart (µg/g)	Bone (µg/g)	Feces (µg/g)
Normal control	4.74 ±	0.50 ±	2.05 ±	0.49 ± 0.07 ^{gh}	0.45 ±	3.28 ±	1.12 ±
G1 (1/4 LD_{50} of Pb)	60.33 ±	23.75 ± 1.61 ^{b}	49.28 ± 2.99 ^{b}	3.40 ±	2.77 ± 0.11 ^{f}	61.97 ± 2.81 ^{f}	60.10 ± 2.46 ^{c}
G2 (1/8 LD_{50} of Pb)	35.16 ± 2.30 ^{b}	13.41 ± 0.74 ^{c}	29.54 ± 1.76 ^{b}	1.96 ± 0.10 ^{c}	1.66 ± 0.12 ^{c}	35.99 ± 1.64 ^{b}	35.79 ±
G3 (1/16 LD_{50} of Pb)	20.67 ± 1.53 ^{d}	7.75 ± 0.64 ^{ef}	19.33 ± 1.51 ^{d}	1.16 ± 0.04 ^{d}	0.97 ± 0.09 ^{e}	21.82 ± 1.47 ^{c}	16.53 ± 0.82 ^{h}
G4 (fed 10% LEP+ 1/4 LD_{50} of Pb)	19.79 ± 1.62 ^{d}	10.35 ± 0.62 ^{d}	18.56 ± 0.81 ^{d}	1.04 ± 0.12 ^{de}	1.52 ± 0.10 ^{c}	25.17 ± 1.13 ^{c}	75.11 ± 3.49 ^{a}
G5 (fed 10% LEP+ 1/8 LD_{50} of Pb)	11.28 ± 0.62 ^{e}	6.36 ± 0.21 ^{f}	10.87 ±	0.66 ± 0.03 ^{fg}	0.91 ± 0.06 ^{e}	14.38 ± 1.81 ^{d}	47.76 ± 1.51 ^{d}
G6 (fed 10% LEP+ 1/16 LD_{50} of Pb)	6.36 ±	3.06 ±	5.91 ±	0.40 ± 0.07 ^{h}	0.52 ±	7.52 ± 0.50 ^{e}	23.10 ±
G7 (fed 10% HEP+ 1/4 LD_{50} of Pb)	31.28 ± 1.34 ^{c}	14.92 ± 0.76 ^{b}	24.28 ± 1.74 ^{c}	2.68 ± 0.21 ^{b}	2.11 ± 0.14 ^{b}	40.03 ± 4.04 ^{b}	69.94 ± 2.38 ^{b}
G8 (fed 10% HEP+ 1/8 LD_{50} of Pb)	18.41 ± 1.09 ^{d}	9.02 ± 0.16 ^{de}	15.03 ± 1.26 ^{e}	1.76 ± 0.14 ^{c}	1.28 ± 0.11 ^{d}	23.99 ± 1.51 ^{c}	40.17 ± 2.02 ^{e}
G9 (fed 10% HEP+ 1/16 LD_{50} of Pb)	11.71 ± 0.88 ^{e}	5.32 ±	9.78 ±	0.82 ± 0.07 ^{ef}	0.97 ± 0.10 ^{e}	15.31 ± 1.01 ^{d}	18.93 ± 1.22 ^{h}
L.S.D.	3.4494	1.4653	2.9341	0.2447	0.2001	4.0132	3.8275

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test.

3.5.Histopathological studies:

3.5.1. Liver:

Histopathological changes of rats administered different doses of lead acetate with or without LEP or HEP are presented in Fig (1), where, liver samples of normal rats group showed normal

hepatic lobule. Liver of rats administered ¼ LD_{50} of LA (positive control 1) showed congestion of hepatoportal blood vessel and edema in the portal tract. Moreover, liver of rats administered 1/8 and 1/16 LD_{50} of LA (positive control 2 and 3) appeared focal hepatic hemorrhage and vacuolar degeneration of hepatocytes. Additionally, liver of rats administered ¼ LD_{50} of LA + fed on 10% of LHP clearly slight hydropic degeneration of some heptocytes. There was vacuolar degeneration of some hepatocytes of rats liver administered ¼ LD_{50} of LA + fed on 10% of HHP. No histopathological changes were observed in rats liver administered 1/8 LD_{50} or 1/16 LD_{50} of LA + fed on 10% of LHP or HHP.

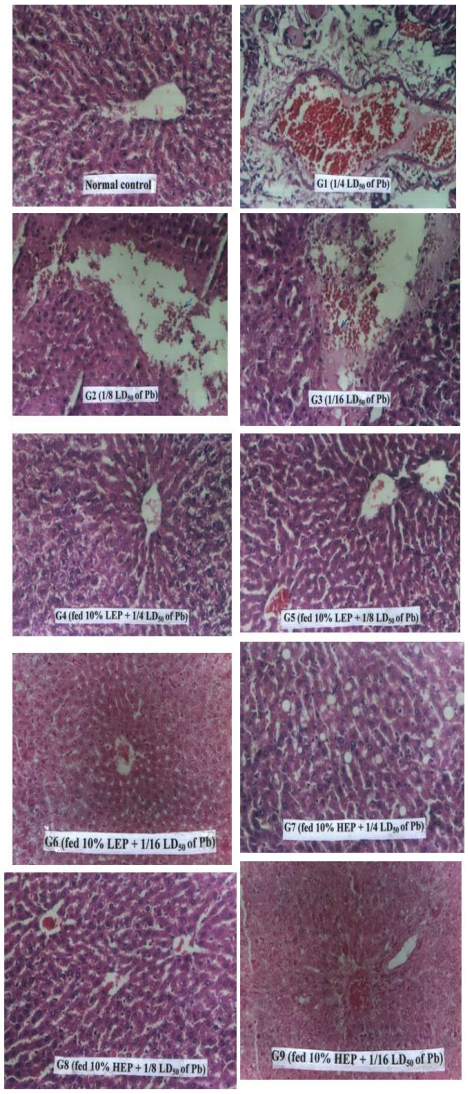

Fig. 1. Histopathological examination of rats liver fed on 10 % LEP or HEP and administrated different doses of lead acetate

3.5.2. Kidney:

Concerning kidney of normal control rats group, it showed the normal histology of renal parenchyma, Fig (2). Kidney samples of group positive control 1 appeared congestion of renal blood vessel and swelling of epithelial liming some renal tubules. Furthermore, kidney of rats administered 1/8 LD_{50} of LA (positive control 2) observed marked dilation and congestion of renal blood vessel. Additionally, there were vacuolation of epithebal lining renal tubules and thickening of the glomerular basement membrane of rats kidney administered 1/16 LD_{50} of LA (positive control 3). All the tested rat groups administered different doses of LA +fed on 10% of LHP or HHP showed no histopathological changes of kidney samples. It could be observed that LHP or HHP had the same effect to protect the kidney tissues from the harmful of lead acetate.

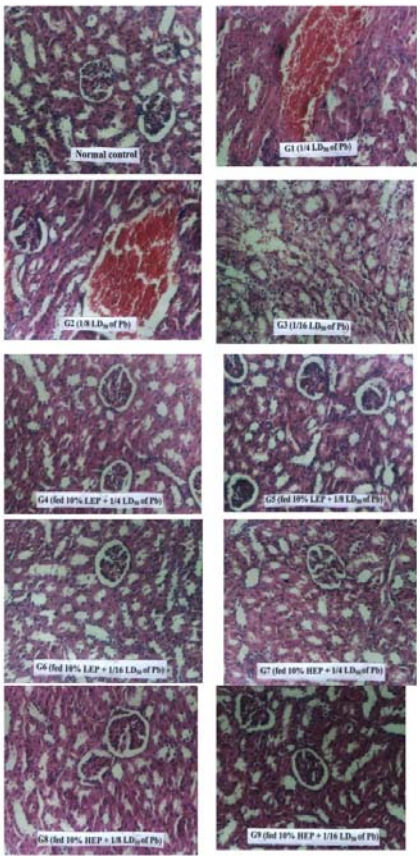

Fig. 2. Histopathological examination of rats kidney fed on 10 % LEP or HEP and administrated different doses of lead acetate

3.5.3.Brain:

Regarding the brain samples of normal control rats group, there was normal histological structure, Fig (3). In contrast, brain of rats from positive control 1 showed neuronophagia of

degenerated neurons. Moreover, there were pyknosis of neurons, focal gliosis and congestion of cerebral blood vessel in brain samples of positive control 2 and 3. The feeding on LHP had protected effect of brain tissues. There was no histopathological changes of brain rat groups fed LEP. Brain of rats administered ¼ LD_{50} of LA + fed on 10% of HHP showed neuronophagia of pyknotic neurons. However, cellular edema was the only change observed in brain of rats administered 1/8 LD_{50} of LA + fed on 10% of HHP. Some examined sections of brain rats administered 1/16 LD_{50} of LA + fed on 10% of HHP showed apparent normal structure.

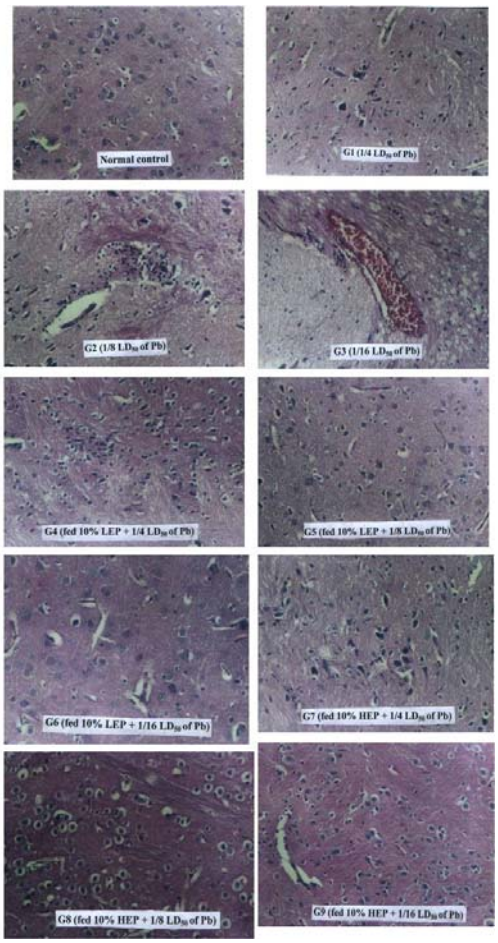

Fig. 3. Histopathological examination of rats fed on 10 % of LEP or HEP and administrated different doses of lead acetate 4.DISCUSION

Lead is a common environmental occupational toxic heavy metal, is known to have direct and indirect effects on biological systems and cells . It is an ubiquitous toxic agent, and its toxicity remains an important public health problem because of the great amount of sources in any household and environment .Its toxicity affecting the nervous system, blood, and blood-forming organs, kidneys, and gastrointestinal tract, Paoliello and De Capitani,(2005).

Our results revealed that the lowest accumulated rat weight gain was observed in positive control rat groups. In addition, there were no significant difference appeared for feed intake of all tested rat groups. At the same time, feed conversion efficiency (FCE) was also decreased by administered oral doses of lead acetate without fed HEP or LEP .This is in accordance to Kang *et al.* (2004) ; Berrahal *et al* (2007);El Nekeety *et al* (2009) ; who reported that mean body weight of the animals treated with LA was significantly lower than that of the other groups. With other organisms also, Hiraga *et al* (2008) exposed young chickens to five lead shot and found that rate of growth was slightly lower in the Pb-exposed group from day 6.

Liver is considered to be the principal target organ for lead toxicity, Abdel-Wahhab *et al.*,(2007).To test for liver function in the present study, results of indicated that HEP or LEP had positive effect on level of serum ALT, AST, ALP and γ- GT activities which were raised by different doses of lead acetate especially at the lowest doses of LA. These results agreed with results obtained by many investigators who studied the effect of lead on liver function; Othman *et al, (*1998); Sivaprasad *et al* (2003) ;Shalan *et al.* (2005) . On the contrary, experiments conducted by Pande *et al*,(2001);Singh *et al.*, (1994) in rats receiving oral administration of lead acetate they reported a significant decrease in the activities of these two enzymes compared to the controls. Studies conducted by Jarrar and Mahmoud (2000); Jevtovic-Stoimenov *et al,(* 2003); Shalan *et al.*, (2005) showed significant increase in the γ- GT activity after a period of Pb exposure for the rats.

Studies in humans have indicated an increase in the activity of these enzymes. Nehru and Kaushal (1993) ;Goswami and Bhattacharya (2000) .Serum total proteins and albumin level, normally used to assess the ability of synthetic function of the liver is well understood and documented ,Adeyemi *et al* (2009). Billirubin is regarded as a member of the antioxidant family, even though it is known to have toxic effects at high concentrations ,Hagymàsi *et al.*, (2003) . It has been regarded for many years as cytotoxic, mainly because of its association with neonate jaundice and its possibility of provoking irreversible brain damage at high concentrations ,Tomaro and Batlle,(2002).

Our results reported that serum billirubin of test rat was found to be about two folds that of control while the serum albumin concentration of test rat was about half that of the control. This result agreed with experiments conducted in rats receiving

lead acetate reported similar decrease in the serum total protein and albumin levels ,Jevtovic-Stoimenov *et al,(* 2003) ,Swarup *et al(,*2007). The decreased level of protein may indicate protein catabolism dysfunction ,Abdel-Wahhab *et al.*,(2008). Moreover, Berrahal *et al* (2007); Adeyemi *et al* (2009) noted a significant increase in the billirubin level (*p* the group of rats treated with Pb compared to control, while serum globulin and albumin concentrations of serum of test rat was significantly lower ($p < 0.05$) than those of control.

Urea and uric acid are the principal waste products of protein catabolism. They are synthesized in the liver from ammonia produced as a result of the de-amination of amino acids ,Young, (1972). Creatinine is the major waste product of creatine metabolism by muscle. In the kidney, it is filtered by the glomerulus and activity excreted by the tubules. Moreover, free creatinine appears in the blood serum ,Baker *et al*, (1979).

There was increase in serum urea, uric acid and creatinine appeared in positive rat groups of our results. Which situation agreed with results of Abdel-Wahhab *et al.* (2007); El Nekeety *et al* (2009); Adeyemi *et al* (2009) studies who illustrated that the exposed to lead increase levels of serum urea may indicate kidney dysfunction than normal control. Scientists explained cause of diseases of the kidney for two consequences; The first is failure to retain substances such as protein, amino acids, sugar, water and ions. The second consequence is failure to excrete substances such as urea, creatinine and the waste products. The presence of lead might have caused impairment of the brush border epithelial cells and making them impermeable to urea and creatinine thereby causing their elevated levels in the blood. The over all effect of this may be impaired kidney function, Oloyede *et al,(*2003).

Serum T3 and T4 levels are considered valuable indicators of thyroid function in animals ,Bruker, (1998); Chaurasia and Kar, (1998). It was clearly from our results that LEP and HEP had a positive effect to keep the level of T3 and T4 in serum nearly within normal especially in group that fed on 10% LEP and administered 1/16 LD_{50} of Pb. In this concern, Wade *et al* (2002) cited that altered serum thyroxine (T4), triiodothyronine (T3) and histomorphology of thyroid gland were recorded following subchronic exposure to a complex mixture of 16 organochlorine chemicals, lead and cadmium in male rats for 70 days. Decreased serum thyroxine (T4) and triiodothyronine(T3) level was also noticed by Pratima *et al,(*1997)in fresh water fish (*Clarias batrachus*) after subchronic exposure to lead and cadmium. Chaurasia and Kar, (1998) noticed that in chicken exposed to dietary lead at 1 mg/kg bodyweight for 30 days ,a significant reduction in T3 level and T4 in their serum . Contradictory reports were also available in human subject on the level of circulating thyroid hormones after lead exposure ,Schantz and Widholm, (2001). In addition, Dundar *et al* (2006) mentioned that long-term low-level lead exposure may lead to reduced T4 level without significant changes in T3 level in adolescents even at low Pb-Blood levels.

The hematological system is the major target of low level lead exposure ,Warren *et al.*, (1998). Therefore, lead exposure induces severe oxidative damage in RBCs by inhibiting heme , hemoglobin synthesis and changing erythrocyte morphology and survival , as a result from direct interaction of lead with RBCs membranes, inducing lipid peroxidation , Leggett (1993) ;Sandhir *et al.*,(1994).

From our results ,it could be noticed that, RBCs and WBCs counts in rat groups fed with LEP were higher than others being fed with HLP in the same concentration of lead acetate. Also, there was a fairly significant difference among all treated rat groups and normal control rats group in Hb. Meanwhile, there was no significant differences in heamatocrite values (PVC) between all treated rat groups and normal control rats group expect G7 (fed on 10 % HEP and administrated 1/4 LD_{50} of lead. In concerning that, Simsek *et al* (2009) found that RBCs and WBCs counts, Hb, PCV and values significantly decreased in rats group exposed to lead acetate compared to the control group. Also, Ancheva *et al* (2003) illustrated that lead cause damage to the erythrocyte membrane resulting in hemolysis or a decrease of blood iron level which may be the cause of decreased concentration of haemoglobine and hematocrit value.Chronic oral lead administration cause the development of hypochromic anemia and hemolytic anemia.

Treating the rats with Pb in the form of acetate led to a considerable increase of accumulation of the metal in the blood, liver, kidney, brain, heart and bones compared with the normal group. O'Flaherty (1991) in accordance, showed that the average Pb concentration in bone of rats administered lead acetate rapidly increased. Timchalk *et al* (2006) exposed the rats to oral gavages doses of 1, 10, or 100 mg Pb-acetate/kg/day daily. They cited that Pb-acetate when administered by oral gavages weres rapidly absorbed, since peak blood Pb concentrations were attained within 30 min to 1 h post-dosing and the Pb rapidly redistributed (within 5-days post-treatment) from the blood into the bone compartment based on the substantial decrease in WBcs and RBC Pb concentration, and the concurrent increase in bone Pb following repeated exposure at all dose levels. Moreover, Adeyemi *et al* (2009) found that possible

damage to the tissues of rats placed on water contaminated with lead (0.015 lg/l). The heart and liver were the last sensitive tissues to lead retention.In addition, all groups of tested animals in the present study, fed on LEP or HEP, showed a significant increase lead concentration in feces compared to the positive control rat groups indicating continuous lead elimination through the digestive tract. In this situation, lead concentration in serum and organs was reduced after pectin administration. Dongowski *et al,(*1999) stated that application of native pectin as well as oligogalacturonic acids increased lead elimination through blood and organs. And addition of rhamnogalacturonic parts of pectin into the lead-enriched diet in rats contributed to slow absorption of lead in rats and enhanced lead excretion with feces during the period of the experiment. Moreover, the structural parameters of pectin influence by its microbial degradation in the intestinal tract. Also, enzymes from intestinal microorganisms involved in pectin fermentation include pectatelyase, polygalacturonase and pectinesterase (Dongowski and Lorenz, 1998 and Tierny *et al*, 1994). Additionally, Serguschenko *et al* (2004) proved that pectin exerts high metal binding activity regarding bivalent metal ions. Drawing of absorption isotherms showed that pectin possesses pronounced affinity to lead ions in comparison to other bivalent metals, and some types of natural pectins may be more effective than chelating agents currently used to manage lead toxicity. In some cases, orally administered pectins contributed to increased retention of heavy metal in tissues (Rose and Quarterman,1987).

Many studies illustrated the effect of degree of methylation on the degradation of pectin in the intestinal tract of rats. Dongowski *et al* (2002) found that Low-methoxyl pectin was fermented faster than high-methoxyl pectins in *vivo* and in *vitro*. Kim *et al* (1978) illustrated that high esterified pectins require large amounts of sugar and low pH values for gel formation. While low- ester pectins form gel with or without sugar in the presence of divalent cations. So that the quantity of metal bound to pectin is determined by the number of free carboxyl groups ,Serguschenko *et al.,* (2007). This could enhance the concentration of lead acetate in feces of rats treated with low esterified pectin than high esterified pectin. Moreover, Kartel *et al* (1999) illustrated that high esterified pectin is characterized by a major part of carboxyl group in galacturonan pattern to be occupied with methyl radicals preventing interaction with metal. Therefore, the lowest binding activity of lead was registered in animals treated with different doses of high pectin sample. Also, using high doses of lead acetate and pectins contributed to a considerably rapid elimination of the lead from the rat body. Fast elimination of metal from tissues as a result of treatment with pectin substances led to redistribution of lead in the body of the animals. This could be confirmed by several fold increases of the metal contents in the liver and heart. This phenomenon is sometimes called “rebound” effect ,Gerhardsson *et al*, (1999).

Through our histological study, it was noticed that there was vacuolar degeneration of some hepatocytes of rats liver administered ¼ LD_{50} of LA + fed on 10% of HHP. There were no histopathological changes observed in rats liver administered 1/8 LD_{50} or 1/16 LD_{50} of LA + fed on 10% of LHP or HHP.

The histopathological examination of the liver tissue of the animals treated with lead showed that, lead(Pb)-induce DNA damage (Fracasso *et al.* 2002; Danadevi *et al.,* 2003 and Xu *et al.* 2003). On the other hand, Shalana *et al.* (2005) found that lead reduced hepatic total RNA content indicating a lower rate of hepatic protein synthesis. Furthermore, El-Zayat *et al.* (1996) reported a decrease in hepatic total protein content in response to lead intoxication. These authors attributed that to a decreased utilization of free amino acids for protein synthesis. In another report, Pagliara *et al* (2003) showed that lead-induced liver hyperplasia followed by apoptosis mediated by oxidative stress in kupffer cells. It could be observed that LHP or HHP had the same effect to protect the kidney tissues from the harmful effect of lead acetate.

Many investigates illustrated that the exposed of experimental rats to lead induced significant histopathological changes of kidney tissues ,Patra *et al.*, (2001) and El-Sokkary *et al*,(2005).

brain of rats from positive control , showed neuronophagia of degenerated neurons. Moreover, there were pyknosis of neurons, focal congestion of cerebral blood vessel in brain samples of positive control. The feeding on LHP had protected effect of brain tissues. There was no histopathological changes of brain rat groups fed LEP. Results clearly showed that LA had a harmful and stressful influence on the hepatic, renal and brain tissues consistent with those reported in the literature ,Nehru and Kaushal,(1993) and Singh *et al.,* (1994).

In conclusion, many chelating agents are currently used to manage lead toxicity. At the same time, the most common, however, are nonspecific and have some adverse effects in humans such as induction of misbalance of essential microelements . Because pectins are both specific and effective in complexion with lead, these compounds may be considered as nutritional products that could be used to decrease lead intestinal absorption, prevention of

lead accumulation, and amelioration of lead toxicity. In the present investigation, LE pectin was contributed to fast elimination of the lead acetate to blood, organs and bones, whereas HE pectin removed lesser amount of lead. However, additional studies in rats and humans are required before developing the pectins as preventive or curative agents of lead exposure and toxicity in humans.

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8/5/2010