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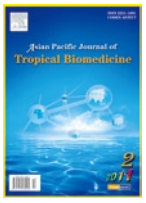

Document heading

Discharge of lead contamination by natural compounds pectin and chitin: biochemical analysis of DNA, RNA, DNase, RNase and GOT in albino rat as an early bio-marker of lead-toxicity

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ABSTRACT

Objective: To study the effect of different concentrations of lead in drinking water on nucleic acid contents, nuclease activities and glutamic-oxalacetic transaminease (GOT) in different tissues and reduce toxic effects of lead on environment especially human and rats by using pectin and chitin natural compounds in rat diets. **Methods:** Male albino rats were divided into eight groups. Groups 1, 4, 5 and 6 were fed on synthetic diet and given deionized water containing 0, 150, 250 and 1 500 μg lead/mL. Groups 2 and 3 were fed on synthetic diet containing 2% apple pectin or 2% grasp shell chitin and served as positive control. Groups 7 and 8 were fed on synthetic diet containing 2% pectin or 2% chitin and drinking water containing 250 μg lead/mL. At the end of the experimental period, animals (6 week) were killed by decapitation. All organs of each rat were dissected out and chilled for determination of DNA, RNA, DNase, RNase and GOT. **Results:** The data showed that higher lead concentration increased the activity of GOT in all organs. The concentrations of both DNA and RNA were increased with decreasing the activities of DNase and RNase. Adding 2% pectin or chitin with lead concentration 250 μg/mL showed discharge of lead, maintained the amount of nucleic acids and activated the related decomposition enzymes. **Conclusions:** Pectin or chitin natural compounds have the ability to chelate to lead and subsequently work as active natural compounds to discharge lead contamination.

1. Introduction

Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in the polluted environment[1]. The excessive amount of pollutants such as heavy metals in animal feed and feed stuffs are often due to human actions and they result from either agricultural or industrial production or through accidental or deliberate misuse[2-5]. There are at least 18 elements that characterize one or more inorganic pesticide, of these elements eight *i.e.* barium, cadmium, mercury, thallium, lead, bismuth, antimony and boron have not been shown to be essential to the growth of animals[6]. Many heavy metals, including Pb, are known to induce over production of reactive oxygen species (ROS) and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membranes[7]. Also, it has been shown to enhance the production of ROS in a variety of cells resulting oxidative stress[8]. ROS are the byproducts of many degenerative reactions in many tissues, which will affect the regular metabolism by damaging the cellular components[9]. Extensive study on oxidative stress has demonstrated that exposure of cells to adverse environmental conditions induces the over production of ROS, such as superoxide radical (O_2^-), H_2O_2 and hydroxyl radical (OH^-) in plant cells[10]. In addition, ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage[11-16]. Traces of lead occur in many rocks in addition to those that are qualified as over lead, thus it finds its way into soil and water and hence into food,

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animals and human tissues even in remote places where there is no use of the metal or its compounds. In spite of its widespread distribution in tissues, there is no indication that it has no beneficial effect, but it causes many problems to the plant, food industry and animal health. Although various countries have established legislation regulating their concentration, they are still sometimes a danger for consumer health[17]. Lead accumulates slowly in the body and even low doses can eventually lead to poisoning. Exposure to lead has been postulated to contribute to elevated blood pressure in humans and animals and has been shown to produce a variety of toxic effects *i.e.* disturbances of the central nervous system, kidney damage, impairment of cardiovascular system and liver functions[18,19]. Therefore, attention has been given recently to potential hazard of lead from different sources including lead based paint, soil, household dust, water supplies, food tobacco smoke and the atmosphere near highways and lead related industries. Managing blood lead levels of less than 10 μg/dL in children and controlling lead poisoning in an adult as well as lead mobilization by pregnancy should be performed[20,21]. The health based guideline for lead in drinking water is 0.1 mg/L[22]. If high levels are detected in a supply, alternative supplies or bottled water may be necessary to protect young children. Characterization of the environmental landscape of Pueblo in terms of heavy metals, and its related population distributions proved the highest levels of lead as its concentrations ranged between 56.6 and 66.5 ppm and exceeded 300 ppm in several of Pueblo's residential communities[23]. Lead in all form is an accumulative poison. Therefore, the biochemical effect of lead was studied. Lead-induced hypertension could increase cardiac reactivity to β-adrenergics and increase the responsiveness of isolated heart to β-adrenergics. The results indicate that low-level of lead (100 ppm) increases blood pressure and both chronotropic and inotropic effects of β-adrenergics[24]. These effects could imply an important role in the pathogenesis of lead-induced hypertension. Whilst, the toxic effects of nickel sulfate were determined on the biochemical and elemental profile of liver in protein deficient rats[25]. Nickel sulfate in the dose of 800 mg/L in drinking water was administrated to Sprauge Dawley which served as normal control as well as protein deficient rats for a total duration of eight weeks. The effects of nickel treatment and protein deficiency when given separately and in combination were studied on rat liver marker enzymes like alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT). Significant increase in the enzymes activity was noticed. Rats exposed to lead acetate in their drinking water (500 ppm) for 14 days with lipoic concentration (25, 25 and 100 mg/kg) elicited a significant increase in bone marrow lipid hydroperoxides and protein-carbonyl contents and decrease in total antioxidants, superoxide dismutase, glutathione peroxidase, glutathione S-transferase and catalase levels with lead ingestion[26]. The ability to retain some metals by their chelating properties and ionic interaction during preparation of drugs or using natural compounds has been studied[27,28]. The present work was aimed to study the effect of different concentrations of lead as lead acetate (150, 250 and 1 500 ppm) in drinking water on nucleic acid contents (DNA and RNA), nuclease activities (DNase and RNase) and GOT in different tissues (brain, hear, liver, lung, muscle, kidney, spleen and testes). It was also attempted to reduce the toxic effect of lead on the environments especially human and rats by using pectin and chitin natural compounds in rat diets.

2. Materials and methods

2.1. Chemicals

All chemicals used for the present study were of analytical grade and purchased from Sigma, USA.

2.2. Experimental protocol

A total number of 80 adult male albino rats (Sproque-Dawely strain) of an average initial weight of 45 g were used. Each rat was raised individually in a well aerated cage under hygienic conditions at the Animal House of the Biochemistry Department, Faculty of Agriculture, Cairo University.

The rats were fed on synthetic diet consisting of casein (2.0%), starch (5.7%), corn oil (10%), wooded fiber (5%), salts mixture (4%) and vitamin mixture (1%). Diet and water were supplied *ad libitum* for two weeks, then rats were divided into eight groups with 10 rats in each group.

Groups 1, 4, 5 and 6 were fed on synthetic diet and given drinking deionized water containing 0, 150, 250 and 1500 μg lead/mL (as lead acetate), respectively. A volume of 2 mL of acetic acid 5% was added to 1 L of each drinking water to prevent lead precipitation.

Group 2 was fed on synthetic diet containing 2% apple pectin (250 grade, BDH) and drinking water containing acetic acid and served as pectin positive control.

Group 3 was fed on synthetic diet containing 2% grasp shell chitin (Sigma) and the drinking water containing acetic acid as described above and served as chitin positive control.

Group 7 was fed on synthetic diet containing 2% pectin and drinking water containing 250 μg lead/mL.

Group 8 was fed on synthetic diet containing 2% chitin and drinking water containing 250 μg lead/mL. All groups were fed on diets and supplied with different water *ad libitum* for 40 days. At the end of the experimental period, animals (6 week) were killed by decapitation. All organs of each rat were dissected out and chilled for determination of DNA, RNA, DNase, RNase and GOT.

2.3. Biochemical analysis

2.3.1. DNA and RNA determination

DNA and RNA contents of rat organ tissues were determined by the method of Astrawrov[29].

2.3.2. DNase and RNase determination

Deoxyribonuclease (DNase; E.C. 3.1.4.5.) and ribonuclease (RNase; E.C. 2.7.7.7) activities of tissue homogenate were assayed spectrophotometrically[30].

2.3.3. GOT determination

Glutamate oxaloacetate transaminase (GOT; E.C. 2.6.1.1.) activity of all tissue homogenate was determined[31].

2.4. Statistical analysis

All tests were conducted in triplicate. Data were reported as means ± standard deviation (SD). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package (Cohort Software, CA, USA). The results with $P \leqslant 0.05$ were regarded to be statistically significant.

3. Results

The continuous discharge of lead pollution into food and water in all world accelerated the investigation on its biological effect. Therefore, it was found necessary to conduct some lead pollution experiments on male albino rats using different concentrations of lead in drinking water as lead acetate in order to evaluate its effect on the biochemical levels of nucleic acids (DNA and RNA), their decomposition enzymes (DNase and RNase) and GOT activities in the rat organ tissues under investigation. The three levels of lead (150, 250 and 1 500 ppm) were simultaneously given in drinking water *ad libitum* for 6 weeks. The contents of DNA and RNA and their decomposition enzymes (DNase and R Nase) and GOT activities and their relative percentage in all rat tissue homogenate were determined in comparison with the control (Table 1 and Table 2). Data indicated that DNA and RNA contents were influenced markedly by lead concentration in drinking water. In all organs, a marked increase in DNA and RNA content was recorded as response to lead concentration in drinking water observed at the three levels of lead. The increase in nucleic acids contents was higher with 1 500 μg/mL drinking water (brain DNA 201.00 ±0.76, RNA 371.00±1.45) than those observed with other two levels of 150 and 250 μg/mL (DNA 188.00±0.54, 197.00±0.62, RNA 331.00±1.12, 353.00±1.19), respectively as compared with control (brain DNA 171.00±0.20, RNA 312.00±0.26). The relative percentage increase in nucleic acid reached its maximum with lead concentration of 1 500 ppm to 174.7% DNA in muscle, 135.8% RNA in heart. It is very important to note the increase of DNA and RNA contents in response to the intensity of lead pollution. Our results suggest that lead treatment is associated with oxidative stress leading to oxidative DNA damage. Therefore, DNA damage in the peripheral blood as well as in different organs could be a suitable early bio-marker to monitor the environmental or occupational lead toxicity. On the other hand, pectin and chitin which have chelating properties were selected in order to chelate lead ions. Each natural polysaccharide was added separately to the diet with concentration of 2%. Data in Table 1 and Table 2 showed that nucleic acid contents of different rat tissues were not influenced by the addition of pectin and chitin without lead treatments. While the addition of pectin and chitin to the diet of rats treated with

Table 1
RNA distribution in different organs of rats treated with different concentrations of lead acetate in the presence of pectin and chitin (μg/100 g tissue) (mean±SD).

Organ group	Brain	%	Heart	%	Liver	%	Lung	%	Muscle	%	Kidney	%	Spleen	%	Testes	%
Control	312.00±0.26^{g}	100.0	162.00±0.13^{h}	100.0	1 217.00±1.80^{g}	100.0	317.00±0.44^{g}	100.0	54.00±0.14^{d}	100.0	410.00±0.65^{g}	100.0	553.00±0.16^{e}	100.0	339.00±0.32^{d}	100.0
Pectin (2%)	318.00±0.35^{e}	101.9	167.00±0.24^{e}	103.1	1 291.00±2.20^{d}	101.6	301.00±0.33^{h}	95.0	50.00±0.13^{g}	92.6	415.00±0.23^{e}	101.2	548.00±0.18^{h}	99.1	336.00±0.20^{g}	99.0
Chitin (2%)	315.00±0.35^{f}	101.0	171.00±0.30^{d}	105.6	1 246.00±2.10^{f}	99.45	331.00±0.88^{d}	104.9	41.00±0.30^{h}	75.9	416.00±0.22^{d}	101.5	549.00±0.15^{g}	99.3	337.00±0.25^{f}	99.9
Lead acetate (150 ppm)	331.00±1.12^{c}	106.1	179.00±0.45^{c}	110.5	1 313.00±3.20^{c}	103.3	335.00±0.89^{c}	105.7	69.00±0.50^{c}	127.8	430.00±0.54^{c}	104.9	581.00±0.26^{c}	105.1	352.00±0.35^{c}	103.8
Lead acetate (250 ppm)	353.00±1.19^{b}	113.1	191.00±0.46^{b}	117.9	1 382.00±3.60^{b}	108.7	351.00±0.76^{b}	110.7	79.00±0.60^{b}	146.3	452.00±0.86^{b}	110.3	603.00±0.30^{b}	109.0	371.00±0.40^{b}	109.4
Lead acetate (1 500 ppm)	371.00±1.45^{a}	118.9	220.00±0.65^{a}	135.8	1 491.00±3.80^{a}	117.3	369.00±1.49^{a}	116.4	84.00±0.85^{a}	155.6	479.00±0.89^{a}	116.8	610.00±0.50^{a}	110.3	393.00±0.45^{a}	115.4
Lead acetate (250 ppm) + pectin (2%)	317.00±0.33^{e}	101.6	165.00±0.25^{f}	101.6	1 251.00±2.20^{e}	98.43	326.00±0.60^{e}	102.8	53.00±0.12^{e}	98.15	399.00±0.39^{h}	97.3	552.00±0.15^{f}	99.8	331.00±0.24^{h}	97.54
Lead acetate (250 ppm) + chitin (2%)	322.00±0.43^{d}	103.2	163.00±0.18^{g}	100.6	1 251.00±1.20^{e}	98.43	322.00±0.42^{f}	101.6	52.00±0.10^{f}	96.29	411.00±0.23^{f}	100.2	555.00±0.25^{d}	100.4	338.00±0.25^{e}	99.7
LSD 5%	1.42±0.00	–	0.64±0.00		4.60±0.00		1.36±0.00		0.75±0.00		0.98±0.00		0.46±0.00		0.55±0.00	

Values with different letters in the same column were significantly different ($P \leqslant 0.05$).

Table 2
DNA distribution in different organs of rats treated with different concentrations of lead acetate in the presence of pectin and chitin (μg/100 g tissue) (mean±SD).

Organ group	Brain	%	Heart	%	Liver	%	Lung	%	Muscle	%	Kidney	%	Spleen	%	Testes	%
Control	171.00±0.20^{f}	100.0	151.00±0.16^{f}	100.0	261.00±0.23^{f}	100.0	151.00±0.20^{f}	100.0	91.00±0.17^{g}	100.0	421.00±0.13^{g}	100.0	651.00±0.22^{e}	100.0	161.00±0.12^{e}	100.0
Pectin (2%)	170.00±0.25^{g}	99.4	152.00±0.14^{e}	100.7	236.00±0.45^{h}	100.8	152.00±0.28^{e}	100.7	93.00±0.15^{f}	102.2	425.00±0.23^{f}	101.0	651.00±0.17^{e}	100.0	160.00±0.08^{f}	99.4
Chitin (2%)	175.00±0.34^{e}	102.3	152.00±0.11^{e}	101.3	270.00±0.55^{d}	103.4	153.00±0.12^{d}	101.3	96.00±0.08^{d}	105.5	433.00±0.31^{d}	102.9	665.00±0.65^{d}	102.9	162.00±0.09^{d}	100.6
Lead acetate (150 ppm)	188.00±0.54^{c}	109.9	161.00±0.30^{c}	106.6	279.00±0.66^{c}	106.9	163.00±0.22^{c}	107.9	121.00±0.46^{c}	133.0	471.00±0.45^{c}	111.9	683.00±0.55^{c}	104.9	172.00±0.31^{c}	106.8
Lead acetate (250 ppm)	197.00±0.62^{b}	115.2	169.00±0.33^{b}	111.9	280.00±0.78^{b}	107.3	178.00±0.57^{b}	117.9	151.00±0.04^{b}	165.9	495.00±0.62^{b}	117.6	701.00±0.60^{b}	107.7	183.00±0.22^{b}	113.7
Lead acetate (1 500 ppm)	201.00±0.76^{a}	117.5	171.00±0.60^{a}	113.3	290.00±0.87^{a}	111.1	185.00±0.66^{a}	122.5	159.00±0.23^{a}	174.7	521.00±0.66^{a}	123.8	731.00±0.68^{a}	112.3	193.00±0.45^{a}	119.9
Lead acetate (250 ppm) + pectin (2%)	169.00±0.36^{h}	98.8	151.00±0.12^{f}	100.0	260.00±0.44^{g}	99.6	150.00±0.14^{g}	99.34	95.00±0.15^{e}	104.4	421.00±0.11^{g}	100.0	643.00±0.57^{f}	98.8	159.00±0.06^{g}	98.8
Lead acetate (250 ppm) + chitin (2%)	179.00±0.44^{d}	104.7	160.00±0.22^{d}	106.0	263.00±0.33^{e}	100.8	141.00±0.29^{h}	98.7	89.00±0.10^{h}	97.8	431.00±0.12^{e}	102.4	649.00±0.46^{g}	99.69	159.00±0.08^{g}	98.8
LSD 5%	0.82±0.00		0.50±0.00		1.00±0.00		0.63±0.00		0.37±0.00		0.67±0.00		0.90±0.00		0.38±0.00	

Values with different letters in the same column were significantly different ($P \leqslant 0.05$).

Table 3
DNase activity in different organs of rats treated with different concentrations of lead acetate in the presence of pectin and chitin (international units) (mean±SD).

Organ group	Brain	%	Heart	%	Liver	%	Lung	%	Muscle	%	Kidney	%	Spleen	%	Testes	%
Control	79.32±0.06^{a}	100.0	39.63±0.07^{b}	100.0	59.89±0.10^{b}	100.0	50.13±0.09^{b}	100.0	40.42±0.06^{a}	100.0	45.60±0.07^{a}	100.0	58.32±0.10^{c}	100.0	48.21±0.12^{c}	100.0
Pectin (2%)	77.33±0.08^{c}	97.5	40.11±0.12^{a}	101.3	60.91±0.14^{a}	101.7	49.31±0.06^{d}	98.4	39.99±0.07^{b}	98.9	43.98±0.11^{d}	96.4	57.26±0.14^{d}	98.2	48.55±0.09^{b}	100.7
Chitin (2%)	79.51±0.07^{a}	100.2	39.50±0.09^{b}	99.7	59.11±0.12^{c}	98.7	51.10±0.11^{a}	101.9	39.72±0.09^{c}	98.3	44.21±0.14^{c}	96.9	58.93±0.11^{b}	101.0	48.61±0.04^{b}	100.8
Lead acetate (150 ppm)	75.81±0.14^{d}	95.6	37.11±0.08^{d}	93.7	56.61±0.11^{e}	94.5	47.61±0.09^{e}	94.9	38.11±0.05^{d}	94.3	42.31±0.15^{e}	92.8	54.11±0.18^{e}	92.8	46.61±0.08^{e}	96.7
Lead acetate (250 ppm)	71.63±0.11^{e}	90.3	35.71±0.11^{e}	90.1	52.11±0.08^{f}	87.0	45.21±0.14^{f}	90.2	35.91±0.09^{e}	88.9	40.21±0.12^{f}	88.2	49.92±0.07^{f}	85.6	44.15±0.09^{f}	91.6
Lead acetate (1 500 ppm)	67.91±0.19^{f}	85.6	33.25±0.13^{f}	83.9	49.3±0.14^{g}	82.4	42.31±0.12^{g}	84.4	33.21±0.10^{f}	82.2	38.33±0.12^{g}	84.0	45.92±0.05^{g}	78.7	41.32±0.07^{g}	85.7
Lead acetate (250) + pectin (2%)	79.42±0.04^{a}	100.1	39.21±0.13^{c}	98.9	58.32±0.07^{d}	97.4	51.09±0.14^{a}	101.9	38.22±0.13^{d}	94.6	44.11±0.14^{d}	96.7	59.31±0.08^{a}	101.8	49.32±0.12^{a}	102.3
Lead acetate (250) + chitin (2%)	78.93±0.05^{b}	99.5	40.22±0.14^{a}	101.5	58.31±0.09^{d}	97.4	49.93±0.08^{c}	99.6	40.31±0.12^{a}	99.7	45.31±0.13^{b}	99.3	57.38±0.23^{d}	89.4	47.91±0.09^{d}	99.4
LSD 5%	0.17±0.00	–	0.19±0.00	–	0.18±0.00	–	0.19±0.00	–	0.16±0.00	–	0.22±0.00	–	0.20±0.00	–	0.16±0.00	–

Values with different letters in the same column were significantly different ($P \leqslant 0.05$).

Table 4
RNase activity in different organs of rats treated with different concentrations of lead acetate in the presence of pectin and chitin (international units) (mean±SD).

Organ group	Brain	%	Heart	%	Liver	%	Lung	%	Muscle	%	Kidney	%	Spleen	%	Testes	%
Control	48.56±0.04^{b}	100.0	29.31±0.04^{b}	100.0	41.31±0.03^{c}	100.0	22.21±0.04^{b}	100.0	36.41±0.04^{a}	100.0	39.32±0.02^{a}	100.0	31.32±0.02^{a}	100.0	22.61±0.06^{c}	100.0
Pectin (2%)	47.32±0.06^{d}	97.3	28.71±0.05^{c}	97.9	42.31±0.05^{a}	102.4	21.61±0.06^{d}	97.3	35.99±0.02^{b}	98.7	38.52±0.06^{b}	97.9	30.21±0.05^{d}	96.5	23.61±0.03^{a}	104.4
Chitin (2%)	48.32±0.05^{c}	99.3	28.35±0.02^{d}	96.7	41.52±0.04^{b}	100.5	20.31±0.02^{f}	91.5	36.42±0.06^{a}	100.0	37.41±0.05^{d}	95.1	30.41±0.03^{b}	97.1	23.51±0.02^{a}	103.9
Lead acetate (150 ppm)	45.99±0.07^{e}	94.4	27.93±0.05^{e}	95.2	38.61±0.08^{e}	93.5	21.11±0.03^{e}	95.0	34.92±0.07^{d}	95.9	37.13±0.06^{f}	94.7	28.22±0.04^{e}	90.1	20.91±0.05^{e}	90.3
Lead acetate (250 ppm)	43.11±0.08^{f}	88.6	25.99±0.08^{g}	88.7	36.91±0.03^{f}	89.4	19.92±0.07^{g}	89.7	33.01±0.05^{e}	90.7	35.91±0.03^{g}	91.3	24.10±0.07^{f}	77.0	18.92±0.11^{f}	83.7
Lead acetate (1 500 ppm)	41.21±0.06^{g}	84.7	23.26±0.06^{h}	79.4	34.51±0.08^{g}	83.5	17.23±0.09^{h}	77.6	31.92±0.07^{f}	87.7	33.91±0.04^{h}	86.2	23.00±0.06^{g}	73.4	17.11±0.07^{g}	75.7
Lead acetate (250 ppm) + pectin (2%)	49.91±0.03^{a}	102.6	27.91±0.03^{f}	95.2	41.32±0.06^{c}	100.0	22.98±0.06^{a}	103.4	35.52±0.08^{c}	97.6	37.31±0.02^{e}	94.9	31.39±0.08^{a}	100.2	22.91±0.06^{b}	101.3
Lead acetate (250 ppm) + chitin (2%)	49.82±0.04^{a}	102.4	30.00±0.06^{a}	102.4	40.91±0.07^{d}	99.0	21.87±0.05^{c}	98.5	36.51±0.06^{a}	100.3	38.35±0.03^{c}	97.9	30.31±0.03^{c}	96.8	22.11±0.05^{d}	97.8
LSD 5%	0.12±0.00	–	0.09±0.00	–	0.10±0.00	–	0.10±0.00	–	0.10±0.00	–	0.07±0.00	–	0.09±0.00	–	0.11±0.00	–

Values with different letters in the same column were significantly different ($P \leqslant 0.05$).

Table 5
GOT activity in different organs of rats treated with different concentrations of lead acetate in the presence of pectin and chitin (international units) (mean±SD).

Organ group	Brain	%	Heart	%	Liver	%	Lung	%	Muscle	%	Kidney	%	Testes	%
Control	73.31±0.09^{h}	100.0	86.01±0.06^{h}	100.0	108.90±0.08^{g}	100.0	73.61±0.06^{f}	100.0	82.63±0.18^{g}	100.0	77.62±0.09^{f}	100.0	86.31±0.17^{f}	100.0
Pectin (2%)	82.69±0.11^{d}	112.8	87.33±0.09^{f}	101.5	108.10±0.07^{h}	99.2	74.81±0.08^{e}	101.6	90.9±0.15^{c}	110.0	91.65±0.17^{d}	118.1	86.39±0.09^{f}	100.1
Chitin (2%)	82.33±0.06^{e}	112.3	96.80±0.12^{d}	112.6	110.51±0.12^{e}	101.5	75.61±0.09^{d}	102.7	86.83±0.20^{f}	105.1	96.91±0.13^{c}	124.9	88.43±0.11^{d}	102.5
Lead acetate (150 ppm)	97.84±0.10^{c}	133.5	100.00±0.11^{c}	116.3	123.22±0.12^{c}	113.1	82.89±0.25^{c}	112.6	88.31±0.11^{d}	106.9	91.53±0.11^{d}	117.9	91.87±0.22^{c}	106.4
Lead acetate (250 ppm)	98.52±0.08^{b}	134.4	116.21±0.17^{b}	135.1	136.92±0.19^{b}	125.7	98.53±0.11^{b}	133.9	105.33±0.17^{b}	127.5	109.32±0.20^{b}	140.8	100.31±0.22^{b}	116.4
Lead acetate (1 500 ppm)	100.31±0.12^{a}	136.8	136.05±0.15^{a}	158.3	172.71±0.21^{a}	158.6	105.61±0.16^{a}	145.5	123.63±0.15^{a}	149.6	141.31±0.25^{a}	182.1	132.51±0.17^{a}	153.5
Lead acetate (250 ppm) + pectin (2%)	77.53±0.05^{g}	105.8	86.41±0.07^{g}	100.5	109.31±0.16^{f}	100.4	72.11±0.06^{g}	97.9	87.51±0.08^{e}	105.9	83.21±0.11^{e}	107.2	82.51±0.12^{g}	95.6
Lead acetate (250 ppm) + chitin (2%)	77.81±0.10^{f}	106.1	91.31±0.09^{e}	106.2	112.63±0.17^{d}	103.4	73.42±0.07^{f}	99.7	86.91±0.15^{f}	105.2	96.81±0.19^{c}	124.7	86.92±0.16^{e}	100.7
LSD 5%	0.16±0.00	–	0.20±0.00		0.25±0.00		0.22±0.00		0.26±0.00		0.28±0.00		0.28±0.00	

Values with different letters in the same column were significantly different ($P \leqslant 0.05$).

250 μg lead/mL (group 7 and 8, respectively) turned the levels of DNA and RNA to nearly normal approximately in all tissues examined, brain DNA control (171.00±0.20), brain DNA 250 ppm (197.00±0.62), brain DNA 250 ppm + 2% pectin (169.00±0.36), brain DNA 250 ppm + 2% chitin (179.00±0.44). RNA contents had the same trends (Table 1 and Table 2). The activities of DNase and RNase were analyzed in eight different tissue homogenate of rats exposed to lead pollution in their drinking water and rats fed on pectin and chitin in their diet which were also exposed to lead pollution.

Data given in Table 3 and Table 4 revealed that DNase and RNase activities were decreased proportionally as response to the increase of lead pollution in drinking water, brain DNase (150 ppm, 75.81±0.14), (250 ppm, 71.63±0.11), (1 500 ppm, 67.91±0.19) compared to brain control (79.32±0.06). The addition of pectin and chitin natural compounds to the diet also reduced the effect of lead pollution and increased DNase and RNase of tissue homogenate to nearly normal values DNase, (250 ppm, 71.63±0.11), (250 ppm + 2% pectin, 79.42±0.04), (250 ppm + 2% chitin, 78.93±0.05) compared to brain control (79.32±0.06). The same trend could be observed in case of the activity of RNase in different treatments. Lead pollution also affected the activity of GOT of all tissues and the obtained results were given in Table 5. The data showed a positive correlation between the intensity of lead pollution in rat's drinking water and GOT activities of all organs under investigation. It was observed that treatments with natural compounds containing pectin or chitin reduced the toxic effect in all organ tissues by reducing GOT activities (brain, 150 ppm, 97.84±0.10), (250 ppm, 98.52±0.08), (1 500 ppm, 100.31±0.12), (250 ppm + 2% pectin 77.53±0.05), (250 ppm + 2% chitin, 77.81±0.10) compared to control (73.31±0.09). From the above data it is of interest to notice that nucleic acid content reached its maximum values in all rat organs in response to the increase of lead pollution from 150 to 1 500 ppm. This may be due to the mutagenic effect of lead where there was a positively linear relationship between lead administration to rats and its accumulation in their tissues.

4. Discussion

Our data suggest that treatment with lead was associated with DNA damage in a dose-dependent fashion in all organs studied which was approved by Arif *et al*[32]. Both brain and kidney are sensitive to lead exposure which affects nuclear

functions. It is mutagenic in rats and mice, and causes chromosome defects and gives rise to multinucleated cells. The nuclear matrix is an operationally defined fraction which should reflect nuclear activities occurring in an insoluble phase, including DNA replication and transcription[33]. On the other hand, lead induces hepatocyte proliferation and subsequent apoptosis in rat livers[34]. Low activity of ATPase and DNase was observed in rats exposed to lead at different doses over a period of 40 days as in agreement with Chowdhury *et al*[35]. After absorption of lead ion, it is diffused from serum into the cells of all organs including liver, kidney, spleen muscles, *etc* and accumulates there[36]. Generally, mutation is followed by an increase in nucleic acid contents where some enzyme activities are increased in response to lead pollution including serum GOT and GPT and GOT of all rat tissues homogenate[37]. Accordingly, lead accumulation might cause an adaptation of certain enzyme systems especially the oxidation reduction system in all organs including nucleic acid and protein (apoenzyme) biosynthesis to prevent the cell from lead toxicity. On the other hand, these increases of nucleic acid contents in response to lead may be due to feedback inhibition of nucleic acid catabolism whereas it was found that accumulation of nucleotide in the erythrocytes may be due to the effect of lead on nucleotide pool[38]. The increase of all tissues GOT activities followed by lead pollution may be due to the changes in overall protein metabolism. Since ATP concentrations of erythrocytes are correlated negatively with lead concentration but AMP concentrations are correlated positively with lead. These results suggest that lead affected nucleotide metabolism and induced a disturbance of the energy production system. Energy changes were consequently decreased in workers who had blood lead greater than 60 μg/100 g[38]. Accordingly, some of the amino acids were determined by an increase of GOT activity and then the keto acids might be converted to glucose for compensation in the energy production system. Conversely the decrease of DNase and RNase activities by lead pollution may be due to the feedback mechanism regulation where the cells inhibit nucleic acid decomposition enzymes and stimulate protein and nucleic acids biosynthesis or may be due to direct inhibition effect of lead accumulation in the cells by combination with SH-group of the protein and enzyme in biological system. Moreover, the features of heat stress protein, androgen-binding protein, cadherin and many other stressor proteins along with ROS and neuro-endocrine mechanism are highly affected by lead heavy metals exposure[39]. The significant increase in DNA and RNA synthesis in all studied of the brain and other organs is characterized by intensive cellular proliferation and differentiation during pre- and postnatal development which probably reflect enhanced metabolism in developing rat brain, and be exaggerated due to repair processes after Pb intoxication. On the other hand, it was interesting to state that chelation effect of the natural pectin or chitin had a reducing biochemical effect on lead pollution in rats. Data indicated that chitin had more protective effect than pectin. The lead-binding capacity of all pectin compounds was the highest within the pH ranging from 7 to 8. The binding capacities and rates of Pb (II) ions by pectin compounds were evaluated. These results obtained through the study suggest that pectin compounds are favorable sobers. The largest amount of Pb (II) ions were bound by pectin with the low degree of esterification[40]. Chitin (poly-N-acetlglucosamine) is general carrying an amino group or anhydrous glucose unit and thus exhibits uptake capacity for metals. The amino group has an electron pair available for chelation and behaves likes a strong Lewis base and therefore can chelate lead. Other available Lewis base is the hydroxyl group in the polysaccharide composition but their complex formation ability is less than -NH, group in chitin. Pectin is bound with lead through the presence of methoxyl (-OCH) and carboxyl (-COOH) groups. The first has an electron pair available for coordination and carboxyl group helps to bind metals. Therefore, our data suggest that lead treatment is associated with oxidative stress-induced DNA damage in all organs studied and could be used as an early bio-marker of lead-toxicity[32]. Finally, pectin or chitin natural compound had the ability to chelate to lead and subsequently works as active natural compound to discharge lead contamination.

Conflict of interest statement

We declare that we have no conflict of interest.

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