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Clinicopathological studies on the antioxidant effect of barley on chicken affected by lead toxicities.

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Forty five, 21-day-old balady chick were used in this experiment. Chicks were divided into three equal groups. The 1st group used as control. The 2nd and 3rd groups were supplemented with lead (Pb) acetate (1500 ppm) in drinking water but the latter group received ration containing 20% barley. Blood samples were collected from the wing vein after 1, 2 and 3 weeks. RBCs count Hb concentration and PCV were significantly decreased in group II during the experimental period. RBCs indices showed a significant decrease in MCH and MCHC in group II after 2 and 3 week of experiment. Leukogram showed heteropenia and moncytopenia. Total protein values showed significant decrease in group II due to decrease in serum albumin level after 2 and 3 week of experiment. Significant increase in activity of liver enzymes AST & ALT and serum uric acid were observed in group II after 3 weeks of experiment. Measurement of serum level of malondialhyde (MDA) revealed a significant increase in group II after 2 weeks of experiment while the total antioxidant capacity (TAO) showed significant decrease in group II after 2 and 3 weeks of experiment. Results of the lead residues in the experimental groups revealed that lead residue in muscle, liver and kidney were rise in group II compared with groups I and III.

Environmental pollution is a major problem for human, animals and birds. Lead causes various toxic effects when introduced into the body by ingestion or inhalation (Neathery and Miller, 1975). Poultry affected by lead toxicities through water contamination with lead arising from industrial activity. Supplementation of lead to chickens food causes decrease in body weight gain, feed conversion and egg production (Berg *et al.,* 1980; Bakalli *et al.,* 1995; Edens and Garlich, 1983). Shibamoto and Bjeldanes (1993); Abd El-Khalek et *al.,* (2000) found that lead poisoning in poultry cause anemia, disturbance in hepatorenal function, muscular pain, and neuropathy of both central and peripheral nervous systems. Recent studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS), reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (Hsu and Guo, 2002).

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It is plausible that impaired oxidant/ antioxidant balance can be partially responsible for the toxic effects of lead (Gurer and Ercal, 2000). Many researchers have investigated the benefit of antioxidants in preventing lead toxicity (Hsu and Guo, 2002). Barley seeds have an effect on scavenging ROS. It may exert the inhibitory effect on hydrogen peroxide (H2o2) by blocking H2o2 induced oxidative DNA damage, cell death and apoptosis (Madhujith *et al.,* 2006; Jeong *et al.,* 2009)

The present work was designed to study the hematological, serum biochemical changes and antioxidant effects of barley in chickens affected by lead toxicity.

Material and Methods

Chickens. Forty fife chickens of 21 day old balady chick were used in this experiment. Chicks were divided into three equal groups. The first group used as control group .The second group was supplemented with lead (Pb) acetate 1500 p.p.m. in drinking water (Bahri *et al.,* 1994). The third group was supplemented with lead (Pb) acetate 1500 p.p.m. in drinking water and received ration contain 20% barley (Tabeidian and Sadeghi, 2006).The birds were kept under observation for three weeks.

Lead (Pb). Lead as lead acetate was kindly obtained from the National Research Center,

Table (1): mean values ± S.E. of erythrogram in different experimental groups of chickens.

Time (Wk)	Group	RBCs ($x10^6$/µl)	Hb (g /dl)	PCV %	MCV (Fl)	MCH (Pg)	MCHC %
1st	I	2.00±0.03	9.14±0.50	27.8±0.30	137.2±2.00	42.63±3.00	33.90±2.00
	II	1.63±0.10	5.47±0.40*	25±0.80	148.19±15.00	37.72±2.00	21.33±2.00**
	III	1.73±0.10	7.43±0.40	26.75±1.00	148.2±4.00	38.85±5.00	31.30±4.00
2nd	I	2.02±0.06	9.16±0.05	28±0.80	138.5±2.00	43.7±3.00	33.00±2.00
	II	1.66±0.04**	6.18±0.80*	25.75±0.70*	148.3±4.00	36.8±1.00	24.60±1.00*
	III	1.92±0.05	8.09±0.60	27.8±0.80	130.8±25.00	40.9±2.00	32.10±4.00
3rd	I	2.03±0.06	9.16±0.50	27.7±0.30	138.5±2.00	42.7±3.00	32.36±1.00
	II	1.57±0.10*	6.59±0.10*	25.2±1.00*	153.6±1.50	34.9±0.60**	22.70±0.30*
	III	1.78±0.10	9.24±0.60	27.6±2.00	151±5.00	40.12±2.00	29.50±1.00

Group I: Normal control. Group II: Lead treated group. Group III: Lead and barley treated group.
* Significantly different from control, $P < 0.05$. ** Significantly different from control, $P < 0.001$.

Dokki, Egypt. It was given at a dose of 1500 ppm in drinking water.

Diagnostic kits. Commercial diagnostic kits were purchased from Spinreact, Diamond, Egypt and Biodiagnostic for determination of hemoglobin (Hb), serum total protein, albumin, aspartateaminotranseferase (AST), alanine aminotranseferase (ALT) activities, uric acid, calcium (ca), total antioxidant capacity (TAO) and lipid peroxide; malondialdehyde (MDA).

Samples. Blood samples were collected from the wing vein after 1, 2 and 3 weeks. Blood samples were divided into 2 parts; the first part was collected on EDTA for determination of erythrocytes (RBCs) and leukocytes (WBCs) count, Hb concentration, packed cell volume (PCV), and differential leukocytic count according to (Feildman *et al.,* 2000). The second part was collected into plain centrifuge tube for serum separation and determination of total protein according to (Henry *et al.,* 1974) , serum albumin according to (Doumas, 1971), aspartate aminotransferase and alanin aminotransferase activites according to (Reitman and Frankel, 1957) , serum calcium level according to (Sarkar and Chanhan, 1967), uric acid level according to (Young, 2001) , total antioxidant capacity according to (Koracevic and Koracevic, 2001) and lipid peroxide (Malondialdehyde) according to (Satoh, 1978) .

Tissue specimens. Tissue specimens were taken from muscles, liver and kidney for lead residue analysis according to (Al-Ghrais, 1995).

Statistical analysis. Collected data from the different groups of chickens were statistically analyzed for the mean and standard error according to (Selvin, 1996).

Results

Haemogram. Results of haemogram in experimental groups of chickens are shown in (Tables 1-2). Results revealed that values of RBCs count, Hb concentration and PCV were significantly decreased in group II of the experiment. Blood indices showed significant decrease in MCH and MCHC (normocytic hypochromic anemia). Leukogram of group II showed heteropenia, moncytopenia and eosinophilia in at the 3rd week of experiment.

Serum biochemistry. Results of serum biochemical parameters in chickens of different experimental groups are shown in (Table 3).

Values of total protein and albumin showed significant decrease in group II at the 2nd and 3rd weeks of the experiment. Activists of ALT and AST and serum uric acid concentration showed significant increase in group II at the 3rd week of the experiment, while group III showed significant increase of AST activity. Values of serum calcium concentration showed non significant changes in the different groups.

Measurement of serum level of lipid peroxide (MDA) revealed significant increased values in group II at 2nd week of experiment. Measurement of serum level of total antioxidant capacity (TAO) showed significant decrease in group II after 2nd and 3rd weeks of the experiment.

Residues analysis. Results of lead residues in muscle, liver and kidney in the experimental groups revealed that lead residue was highly significantly increased in group II after the 1st and 3rd weeks of the experiment in comparison to group I and III. Significant increase in lead residue in group III was observed in the liver and kidney after one week of the experiment (Table 5).

Table (2): Mean values ± S.E. of leukogram in different experimental groups of chickens.

Time (Wk)	Group	WBCs ($x10^3$)	Differential leucocytic count %			
			Lymphocytes	Heterophils	Monocytes	Eosinophils
1st	I	28.25±0.80	60.60±1.0	35.00±0.50	5.00±.06	0.30±0.01
	II	24.25±0.20	64.00±4.0	32.60±1.00	3.10±0.20	0.50±0.10
	III	26.25±2.00	63.80±1.0	32.60±1.00	4.80±0.10	0.40±0.10
2nd	I	28.00±0.50	61.50±1.0	33.00±0.50	5.25±0.50	0.20±0.10
	II	22.00±1.00	66.00±2.0	31.70±3.00	2.00±0.30*	0.60±0.20
	III	27.00±3.00	61.50±1.0	33.60±2.00	5.00±0.40	0.40±0.02
3rd	I	28.30±0.70	60.00±1.0	36.25±0.80	4.80±0.10	0.30±0.02
	II	23.00±3.00	65.50±2.0	30.50±1.00*	2.25±0.10*	1.60±0.10*
	III	25.00±0.80	61.00±2.0	34.30±2.00	4.00±0.20	0.50±0.20

Group I: Normal control. Group II: Lead treated group. Group III: Lead and barley treated group.
* Significantly different from control, $P < 0.05$

Table (3): Mean values± S.E of some serum biochemical parameters in different experimental groups of chickens.

Time (Wk)	Group	Total Protein (g/d)	Albumin (g/d)	Globulin (g/d)	A/G ratio	AST Ų/l	ALT Ų/l	Uric Acid (mg/dl)	Calcium (mg/dl)
1st	I	3.82±0.20	1.95±0.07	1.86±0.20	1.03±0.10	77.09±2.00	21.16±2.00	5.47±0.90	3.55±0.05
	II	3.00±0.50	1.36±0.30	1.63±0.40	0.82±0.10	77.60±2.00	22.40±2.00	5.03±1.00	3.19±0.40
	III	2.73±0.20	1.34±0.10	1.27±0.30	1.12±0.20	77.98±3.00	21.90±1.00	5.34±0.40	3.80±0.80
2nd	I	3.66±0.40	1.88±0.10	1.74±0.10	1.08±0.20	77.41±5.00	21.33±0.90	5.69±0.60	3.55±0.05
	II	2.16±0.30*	0.85±0.09*	1.31±0.10	0.63±0.20	87.53±6.00	22.02±1.80	10.05±0.20**	4.55±0.90
	III	3.50±0.20	1.76±0.30	1.72±0.20	1.02±0.10	81.66±3.00	20.39±1.00	6.82±0.80	2.75±0.30
3rd	I	3.71±0.20	1.9±0.10	1.79±0.10	1.06±0.20	78.20±2.00	21.66±1.20	5.69±0.60	3.25±0.30
	II	2.42±0.20*	0.89±0.10*	1.52±0.10	0.85±0.01	106.00±4.00**	28.33±1.206*	9.82±0.20*	2.74±0.70
	III	2.95±0.03	1.6±0.10	1.32±0.20	1.20±0.20	85.80±3.00	22.43±1.00	5.01±0.80	4.77±0.80

Group I: Normal control. Group II: Lead treated group. Group III: Lead and barley treated group.
* Significantly different from control, $P < 0.05$ ** Significantly different from control, $P < 0.001$

Table (4): Mean values± S.E of serum lipid peroxide and total antioxidant capacity in different experimental groups of chickens.

Time (Wk)	Group	Lipid peroxide (nmol/ml)	Total antioxidant capacity (mmol/l)
1st	I	10.15±3	1.79±0.2
	II	10 ± 2	1.48±0.1
	III	10 ±1	1.8±0.1
2nd	I	10. 53±2	1.6±0.1
	II	15. 55±2*	1.2±0.4
	III	8.13±0.8	2±0.04
3rd	I	9.64±0.6	1.69±0.2
	II	13. 28±0.3	0. 89±0.1*
	III	7.24±1	1.72±0.1

Group I: Normal control. Group II: Lead treated group. Group III: Lead and barley treated group.
* Significantly different from control, $P < 0.05$

Table (5): Lead concentration (p.p.m.) in muscle, liver and kidney of chickens in different experimental groups.

Time (Wk)	Group	Muscle	Liver	Kidney
	I	0. 42±0. 04	0.7±0. 2	0.6±0. 1
1st	II	1.69±0. 1*	6.41±0. 2**	5.29±0. 3**
	III	1.2±0.02	4.9±0. 02*	4.6±0.01*
	I	0.41±0.01	0.8±0.1	0.7±0.05
3rd	II	4.7±0. 4**	9.25±0. 4**	7.63±0. 6**
	III	2.6±0. 1	4.1±0. 2	3.9±0.8

Group I: Normal control. Group II: Lead treated group. Group III: Lead and barley treated group .
* Significantly different from control, $P < 0.05$ ** Significantly different from control, $P < 0.01$

Discussion

Results of the erythrogram showed normocytic hypochromic anemia that agree with findings of (Bassiouni *et al.,* 1998; Abd El-Khalek *et al.,*2000; Khan *et al.,* 1993).The decreased erythrocytic count, Hb concentration and PCV in group II could be attributed to the shortened life-span of the red cells. In addition, lead showed a direct effect on aminolevulinic acid (ALA) dehydrase enzyme that share in the synthesis of heme (Webb, 1977; Hermes, 1991; Youssef *et al.,* 1996). Studying of differential leukocytic count revealed heteropenia, monocytopenia and eosinophilia in group II after 3 weeks of the experiment .This may be due to the toxic effect of lead (Khan *et al.,* 1993). The significant decrease in serum total protein and albumin and non significant alteration in A/G ratio in group II may be due to toxic effect of lead or could be attributed to reduction in feed consumption and hepatic damage by lead as the liver is the major organ of protein synthesis specially albumin, (Kaneko *et al.,* 1997) The obtained results agree with (Abd El-Khalek *et al.,* 2000). Significant increase in AST, ALT activities and uric acid in group II after 3 weeks of the experiment may be attributed to toxic hepatitis and nephropathy that were known under condition of lead poisoning (Jones and Hunt, 1983).

The liver and kidneys are also known to play a major role in the elimination of lead (Goyer and Chirian, 1979). Analysis of lead residue in the liver and kidney of this group coincided with these results and agree with (Brar *et al.,* (1997; Menha *et al.,* 2000; Khaled *et al.,* 2008).

The results of our study revealed increase in lipid peroxidation (MDA) in group II in comparable with control group. These results are in agreement with (Jiun and Hsien,1994; Kasperczyk *et al.,* 2005; Emrah *et al.,* 2007; Khaled *et al.,* 2008). This may be due to the toxic effect of lead on erythrocytes and increasing MDA concentrations (Bechara, 1997; Quinlan *et al.,* 1988).

The parameters of haemogram and serum biochemical analysis in group 3 were within that of the control group. This may be due to the antioxidant effect of barley in the treatment of lead toxicities (Gurer and Ercal, 2000; Jeong *et al.,* 2009). Measurement of MDA revealed increased values in group II at second week. MDA is the end product of lipid peroxidation, increase in its level indicates oxidative stress due to the toxic effect of lead (Emrah *et al.,* 2007; Khaled *et al.,* 2008). Significant decrease in the serum total antioxidant level (TAO) in lead treated group was recorded. This result was in agreement with (Hsu, 1981; Sivaprasad *et al.,* 2003; Emrah *et al.,* 2007; Khaled *et al.,* 2008) .This may be due to oxidative stress of lead with generation of reactive oxygen species, reducing the antioxidant defense system of cells via interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. The values of MDA and TAO in barley treated group III were within that of control group that could be attributed to the antioxidant effect of barley (Jeong et al ., 2009).

Analysis of lead residue in muscle, liver and kidney revealed significant increase in group II in comparison to group I and III that agree with results obtained by (Bahri *et al.,* 1994; Abd El-Khalek *et al.,* 2000). Residue analysis in group III in the studied organs was lower than that of group II after the 3rd week of experiment. This may be due to the antioxidant effect of barley in reducing the lead toxicity (Gurer and Ercal, 2000; Hsu and Guo, 2002; Abdel-Dayem, 2004).

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دراسات باثولوجية إكلينيكية على التأثير المضاد للأكسدة في الشعير في الدواجن المصابة بالتسمم بالرصاص

تم تقسيم خمسة وأربعون من الكتاكيت البلدية عمر ثلاثة أسابيع إلى ثلاثة مجموعات متساوية ، المجموعة الأولى تم استخدامها كمجموعة ضابطة ، و تمت إضافة خلات الرصاص في مياه الشرب لكل من المجموعة الثانية والثالثة بنسبة 1500 جزء من المليون مع إضافة الشعير فى العليقة بنسبة 20% للمجموعة الثالثة فقط وتم تجميع عينات السيرم أسبوعيا علي مدار ثلاثة أسابيع وأظهرت النتائج في الأسبوعي الثاني والثالث نقصاً معنوياً فى عدد كرات الدم الحمراء و نسبة الهيموجلوبين وحجم الخلايا التكثفى و عدد كرات الدم البيضاء للمجموعة الثانية كما أظهرت الاختبارات البيوكيميائية زيادة فى إنزيم أ . س . ت . ، أ . ل . ت . وزيادة فى حمض البوليك لنفس المجموعة ، وبدراسة البروتين وجد نقص معنوي فى الألبيومين وزيادة فى المالوندهيد ونقص فى قدرة مانع التأكسد الكليّة للمجموعة الثانية. أما المجموعات المعالجة بالشعير فأظهرت تحسناً واضحاً فى هذه النتائج وبقياس متبقيات الرصاص في العضلات والكبد والكلي وجد زيادة ملحوظة في المجموعة الثانية تحسناً في المجموعة الثالثة مقارنة بالمجموعة الضابطة.