Reduction of Protoporphyrinogen IX oxidase (PPOX) is a key role in lead acetate anemia.

[1]D. S. Dheyab , [2]Majeda AJ Alqayim

[1]Department. of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University.

[2]Department. of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University.

[1] dena.s269@covm.uobaghdad.edu.iq.

Orcid : https://orcid.org/0000-0002-2292-9289.

Corresponding authors :

.[2] dr.majeda@covm.uobaghdad.edu.iq.

Orchid: https://orcid.org/0000-0003-3398-4547.

ABSTRACT

Lead as one of the environmental pollutants can threats the life of living creatures in many ways. It is a poisonous metal and ecological pollutant famous to health of human and animal from long time .The goal of this ready labor was to Clarify type of anemia by influence on complete blood picture , some biochemical tests , PPOX enzyme and clinical signs that induced experimentally by lead.A sixteen local rabbits were divided into two groups, each group have 8 rabbits, control negative were taken food and water only and positive group given lead acetate at 10 mg/ kg/day for 30days.Gathering blood samples to analyze hematological and biochemical tests . After 4 weeks of exposure lead acetate the results showed a significant reduction in Hb ,pcv, MCV, MCH, MCHC ,body weight ,ppox and RBCs counts .From another side a significant increase in RDW and bilirubin concentration were looked . From this study that the decreased in red blood cells indices and hemoglobin concentration led to anemia in animal groups that had administered lead acetate 10mg/kg/Bw.

Keywords : Bilirubin, Hb , lead acetate ,ProtoporphyrinogenI Oxidase ,RBCs indices.

INTRODUCTION

Anemia is a blood trouble in which the blood has a reduced ability to carry oxygen due to less than normal number of red blood cells, or lowering in the amount of hemoglobin. It is a common nutritional deficiency disturbance and global public health problem **(**Soundarya,2016). Anemia has that its seen consequences go beyond reduced physical capacity to affect abroad spectrum of physiologic function (Al Saedi1,2021; ATSDR,2007) . Many types of anemia

classification are found . The occurrence of anemia is due to the various red cell disorder like production , maturation, hemoglobin synthesis , genetic of hemoglobin maturation defect and physical loss of red cells and finally iron deficiency anemia(AL Jabbar ,2015, Mukherjee and Ghosh ,2012**).** It is mainly expected that about 50% of the cases of anemia are due to iron deficiency, but the proportion may fluctuate among population groups and in different areas according to the local situations**(**Zeki and Al-Warid,2019**)**.Protoporphyrinogen oxidase PPOX is consider one of the essential enzymes that synthesis of the hem, as staple of hemoglobin and hemoproteins. It is responsible for the seventh step in biosynthesis of protoporphyrin IX**(Frank *et al*,1999)**. It is a factual internal mitochondrial membrane protein and requires molecular oxygen and the flavin adenine dinucleotide (FAD) (Albert ,2021).When decrease the (PPOX) porphyrins are oxidized to protoporphyrin and coproporphyrin, brought in plasma of the blood and cause increase in sensitivity of the skin to sun light .It possibly related with skin symptoms and neurological symptoms . **(** Mohammad ,2022).Porphyria is typically is the term describe the hyperporphyrinemia, caused by defect in PPOX is suspected in animals because of cutaneous photosensitivity and/or discoloration **(Andrea, 2022)**. Hereditary PPOX deficiency or insinuation to heavy metals can lead to sideroblastic anemia a combined with porphyria **.** The inhibition of this enzyme is a strategy used in certain herbicides, proto supervene (ROS) that's lead to lipid peroxidation(Dayan and Hamed,2015).

Lead(pb) is a heavy metals gray color, its comes from the Latin word Plum bum and it occurs at the top of the earth naturally(Ashour,2022; Salman and ,Dawood,2021). The accumulation of heavy metals by crops has been regarded as a severe environmental hazard because significant accumulation of Pb toxicity where wastewater irrigation has become a common practice **(**Shahzad A ,Zafar,2021**)**, this kind of irrigation is common in Iraq. A Products of lead are felled pollutants in the ambience as well as being created by industrialization and mining action**(Abdulmotalib J*et al*.,2016).** It promote production of reactive oxygen species ROS that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems (Carmouche *et al*,2005 ;Contaldo *et al*,2019**)**.The effects of lead acetate on the hematologic system as a toxic agent with multiple target organs such as, , kidneys ,immune, gastrointestinal and nervous system (Durgut *et al*,2008) , a heavily leading to hypochromic normocytic (microcytic) anemia **.**Neurotoxicity of lead acetate was acknowledged in human and animal species, the degree of influence depended on the concentration of the toxic dose (Jwad *et al*,2012**).** In liver cells in vitro, that cause direct DNA damage and mutation (Jwad *et al.*2012l; Sharma,2010**).**Lead is hurtful , because it generally added to the else metals (e. g , zn, ca, and Fe) in the biological processes. Another metals displacement of intervenes with proteins that organize gene expression disturbances , and hemoglobin biosynthesis **(.Kumar,2022).** The present study aimed to elucidate interfere of lead with PPOX enzyme and the induction of anemia.

MATERIALS AND METHODS

Animals and experimental design

Sixteen healthy rabbits with age 6 months and female gender were distributed into two groups and placed in animals residence of veterinary medicine from 28 may to 28 June 2022 , each of them groups have:.

Group 1: control negative group (8 animals). **Group 2** :positive group(8 animals): this group was treated with lead acetate at 10 mg/ kg per day for 30 days. These animals were then scarified and samples of blood were taken from these heart to prepare :

hematological and biochemical analysis.

Body weight gain obtained from the differences between the initial weight and the final weight

Complete blood count (CBC) was determined by an automated hematology (Genex,USA) .

Biochemical study

Total bilirubin concentration was specified via laboratory kit purchased from (**bio lab-France**) using enzymatic colorimetric methods .

Protoporphyrinogen oxidase enzyme (PPOX) was examined by used Rabbit (Protoporphyrinogen Oxidase) ELISA kit (Elik biotechnology).

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way, two-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $p< o.o5$ is deemed statistically considerable. **(SAS,2010)**

RESULTS

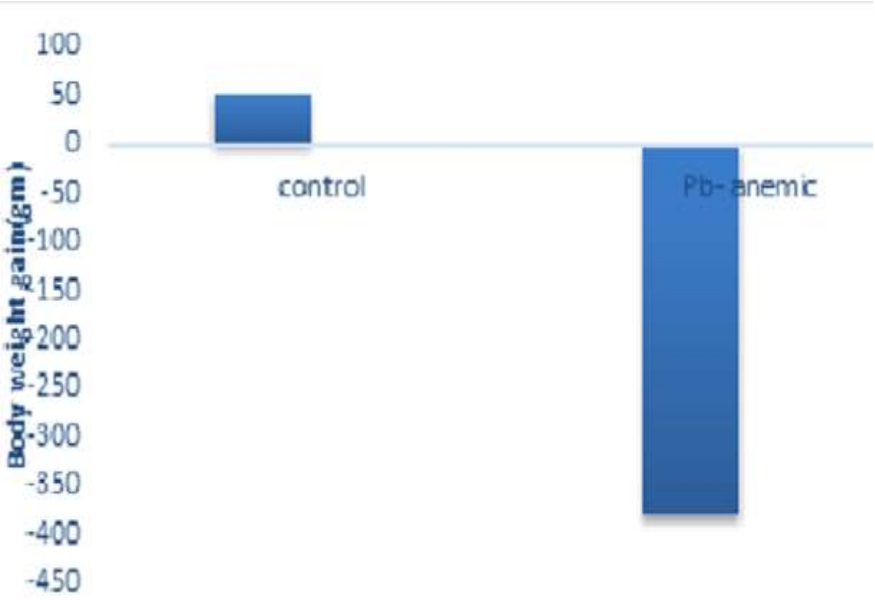
Table 1 in results pointed to belongings oral administration from (Pb)in initial (zero time) and final (30 days) on body weight and body gain in female rabbits ,however non-significant $p>0.05$ changes within value at the first period control and anemic groups ,while decrease significantly $p<0.05$ (1109.38±53.01) in the final period after 30 days of exposure lead acetate were observed compared with control group (1546.88±11.01) .The mean value of body weight gain in figure (1) obvious at the end of experiment were (-381.3±49.94)respectively .

Table 1. Body weight (gm.) in negative and anemic groups

Groups	**Zero time**	**30days**
Control -	**A1493.75±12.27a**	**A1546.88±11.01a**
Anemic	**A1490.63±14.12a**	**B1109.38±53.01b**
LSD	**82.98**	

significant differences (p<0.05) denoted by unlike big letter in the same row and diverse small letter in the same column.

Figure 1. Body weight gain (gm) in control And Pb-anemic rabbits



Hematological and biochemical parameters

The results in table (2) explained the hematological parameters in control and treated group. There are significant p<o.o5 decreased in RBCs, PCV, Hb, MCH,MCV ,MCHC in Lead acetate group compared with control and zero time group , table (2,3) also which showed elevation in parameters of serum bilirubin concentration and RDW p<0.05 significantly in lead acetate are a good indicator to anemic comparison with zero time and control group.

Table 2. Hematological parameters in negative and anemic groups

		RBCs	**PCV**	**HB**	**MCH**	**MCV**	**MCHC**	**RDW**
Control	**zero**	A5.19±0.05a	A36.98±0.07a	A12.23±0.12a	A27.23±0.12a	A82.20±0.46a	A33.83±0.18a	A12.52±0.17a
	30days	A5.20±0.05a	A36.97±0.07a	A12.17±0.12a	A27.12±0.11a	A82.00±0.56a	A34.16±0.20a	A12.45±0.23b
Anemic	**zero**	A5.21±0.05a	A36.91±0.08a	A12.18±0.13a	A27.11±0.12a	A82.22±0.64a	A33.85±0.19a	B12.36±0.23a
	30 days	B4.25±1.15b	B31.72±0.58b	B8.86±0.53b	B19.98±0.53b	B64.37±0.53b	B30.62±0.10b	A17.80±0.53a
LS		0.26	0.87	0.84	0.83	1.60	0.55	0.94

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Significant differences (p<0.05) denoted by different capital letter in the same row and distinct small letter in the same column.

Table 3. Total serum Bilirubin concentration mg/dl in control and Pb- anemic groups

Groups/Bilirubin	**Zero time**	**30 days**
Control -	A0.21±0.02a	A0.21±0.03b
Anemic	B0.23±0.03a	A0.32±0.04a
LSD	0.09	

Significant differences (p<0.05) denoted by diverse capital letter in the same row and different small letter in the same column.

Table (4) illustrate the value of protoporphynogen oxidase (ppox) in control and anemic groups .The result recorded decrease significantly p<0.05 (0.23±0.02) of ppox in the final period 30 days compared with control group (0.79±0.03), while non-significant p>0.05 changes in the zero time in control and anemic groups were seen

Table 4. Protoporphynogen oxidase (nmol/mL in control and Pb-anemic groups.

Groups/PPOX	**Zero time**	**30 days**
Control -	A0.78±0.03a	A0.79±0.03a
Anemic	A0.80±0.02a	B0.23±0.02b
LSD	**0.07**	

Significant differences (p<0.05) denoted by different capital letter in the same row and unlike small letter in the same column.

DISCUSSION

In the current study the insinuation female rabbits to lead acetate for 30 days was searching. The exposure dosage were produced a cumulative effects on hematological system and disorder in body weight . The current results reverberated the hurtful effects from Lead exposure on the rabbits health certain by the lessening of body weight, the final bodyweights of anemic animals

were significantly least compare with the hygienic controls. Results are in a agreements with second study, which found that fed on pb caused depression in growth rate **(**Seddik *e t al*.2010**; Ibrahim *etal*.,2011; Nabil *et al* .2012**). These result s about a body weight gain supposedly caused the toxic ions and could be related with varied factors that created imponderables metabolism and by macerating zinc status in zn-dependent enzymes ,which are needful for plentiful metabolic processes.

The worthy lowering of MCH , MCHC and MCV caused by lead for 30 days might be due to lowered PCV value and Hb , RBCs production and showed the lowering in RBCs count ,pcv % because that bone marrow could overcome lead acetate and led to toxicity(**Rania,2008).**. An increase in the value of RDW. It has been supposed that rise in (RDW) could theorize an caused inflammation, oxidative stress, anemia, and proposed that retard erythrocyte clearance, thereby raised RDW, could be a a stress and poor health that's results from physiological reaction of the body .(**Weiss G and Good,2005).** This could potentially explain the relevance between RDW ,mortality and the connection with poor estimate in many various groups. .From other side the plasma bilirubin concentration increase value down the treatment with lead perhaps the creation of hem oxygenase. Breakdown a hem from whole proteins hem executed in a microsomal portion cells via a complicated enzyme arrangement it competence modify hem into bilirubin (Seddik *et al*.2010**)** they recorded its created within several tissues is imparted to Liver as a compound together S.Bil. It is combine together glucuronide in SER of liver, however the conjunction of bilirubin with glucouronoid will turn into inefficient and possibly lead to peroxidation the lipid membrane of (SER) below the toxic effective of Pb..

The reduction in serum level of protoporphinogen oxidase (PPOX) in the present study, further high light another toxic effects for Lead acetate. Eukaryotic PPOs have Flavin Adenine Dinucleotide (FAD), a non-covalently related co-factor that employ (O2) as the final received an electrons and is needful for PPO action (Koch *et al*.2004) This enzyme expression can be influenced at gene expression by the finite accessibility of the intra-cellular FAD Cofactor probable (negatively) affected and at post-translational modifications increased oxidative stress and an increased presence of reactive oxygen species with Pb is characterized by a decreased intracellular glutathione (GSH) concentration (,Mahmood a nd Al-Helaly .2021; Qu M *et al* 2022**)** due to increased cytosolic calcium ions (Ca2 +) as mentioned by **(Peng and Jou,2010)**'It is listed that lead acetate resort to localize in the mitochondria and destroy mitochondrial function, causing undue mitochondrial ROS generation. The cumulative ROS could hurt mitochondrial function and elevation in ROS generation, that at last leads to mitochondrial impairment . Mitochondrial dysfunction resulted from Pb (.Martin K and Norbert,2022**; Miriam *et al*.2021)** influence PPOX function and hem synthesis (Kanungo *et al* .2018) Also, lead has big leverage on pathways ,such as hem synthesis, there by leading to the outset of anemia **(Schumacher,et.al,2000)** Displacement of other metals interferes with proteins that hold gene expression and nervous system disruption disturbances of hemoglobin biosynthesis and anemia, **(.Kumar,2022).** Trace elements play an important and vital role in metabolism, health and disease(Dhahir and Hemed,2015)

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