



## THE THIAMINE (B1) EFFECTS ON SOME BLOOD PARAMETERS IN LEAD POISONING MALE RABBITS

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### ABSTRACT

The present investigation has been determined the some hematological parameters changes following the lead acetate toxicity and the collaboration of thiamine (vitamin B1) in calming of these changes in male rabbits. Thirty local breed male were used and randomly partitioned into three equivalent groups (n=10). Group G1 served as control, took distilled water, group G2 reserved lead acetate (5 mg/kg B.W), group G3 reserved thiamine (100 mg each animal with lead acetate (5 mg/kg B.W), orally every 24 hours (for 6 weeks), the samples were collected randomly from four animals at time intervals through 1, 3 and 6 weeks. The results revealed significant decline of RBC count, Hb concentration and PCV percentage. Whereas, significant increase of total WBC count and neutrophil count, and in addition there are significant decline of lymphocytes. On other hand, the outcomes demonstrated that splashing vitamin B1 had an essential effective in enhancing the harmful impacts of lead acetate. It can be concluded that, the exposure to lead compounds leads to many distinct alternation in blood tissue due to toxic effects, also, proved that thiamine have an important efficient role in improving the toxicological changes resulted from lead acetate poisoning.

**Key Words:-**Lead poisoning, Thiamin, Rabbits, blood tissue, Neutrophil.

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### INTRODUCTION

Lead happens normally in nature. However, the greater part of the abnormal states found all through the environment from human activities. Studies indicate that exposure to lead compounds, as a result of its bioaccumulation potential, while, the targets were different tissues and organs of the body, especially those that accumulate at relatively high concentrations (Rubin

& Strayer, 2008).

Some studies have been shown, that the exposure to lead, lead to impair in the lipid and protein properties of liver, in addition to the disruption of the cell membrane with the decline of the liver function in chickens (Aykin-Burns *et al.*, 2003; Upasani *et al.*, 2001).

The researchers also showed the toxic effect of lead on the urinary system, which is caused by the dysfunction of the urinary tubules and kidney glands in addition to the histological changes which vary in severity depending on several factors, which may develop in acute cases to renal failure (Heidari *et al.*, 2002)

Anemia is one of the oldest symptoms associated with lead poisoning due to its effect on the necessary enzymes in the hemoglobin synthesis chain, as well as its effect on Erythrocytes life span, due to its fragility, reduced cell count in the bone marrow and reduced ability to produce new cells (Slobozhanina *et al.*, 2005). As well as a range of differential effects in the reproductive organs of both sexes and the central nervous system (Hertz-Picciotto, 2002).

The seriousness of the problem and its complexity with diversity of its effects, helped the

researchers to training to find ways to solutions for lead contamination and find treatments to reduce the toxic effects of the lead in the body organism. (Markowitz, 2000 ).

Recent studies have turned to the use of natural substances, especially vitamins, after revealing that its role played importance in the life space (Barker, 2002 ).

Vitamin B1 is one of the vitamins that have multiple physiological effects. Although its physiological mechanism is not fully known, most of the vital functions of this vitamin are due to its effect on the antioxidant agent, which plays an important role in preventing lipids peroxidation, and reducing free radicals and other oxidizing agents like superoxide radical and hydroxyl radical ,hydrogen peroxide, lipid peroxide, which are from the after of lead exposure, which reduce the cells antioxidant defense (Flora *et al*, 2004). Therefore, this study was founded to explore the protective role of thiamin against,side effects of lead acetate on hematological parameters of local rabbits for six weeks.

## MATERIALS AND METHODS

### Animals

Thirty healthy local male rabbits were brought at age of about 2-3 months old, weighing  $1.350 \pm 250$  g, were used in this study. The animals were kept in individual specialized for rabbit and closed tightly of the animal house of Veterinary College, Baghdad University, and were permitted access to water and standard laboratory pellets *ad libitum*.

### Experiment design

After one-week, rabbits were randomly divided into three groups, G1, G2 and G3; with 10 rabbits in each group. The animals in group G1 served as the control and received the distinct dosage of distilled water. The animals in group G2 were treated orally with 0.5 mg lead acetate powder/Kg/ b.w dissolve in 5ml distal water for each animal . Group G3, animals received simultaneously lead acetate 5 mg /Kg/ b.w and thiamine (100 mg each animal . Experiment design was followed for each 48 hours.

### Blood sampling

Blood samples were prepared from the heart of the rabbits (used four animals randomly from each group) directly by using disposable sterilized syringes and transferred into tubes content included EDTA as the anticoagulation agent , at the outset of the experiment (1<sup>st</sup> week) and was followed on the 3<sup>rd</sup> and 6<sup>th</sup> week . For hematological parameter.

### Hematological factors

Common procedures (Jain, 1993) were used for measuring total erythrocytic counts and total leukocytic counts were determined by using of Haemocytometer

(Neubauer counting chamber), packed cell volume (PCV) were determined by a microhematocrit centrifuge ( $12,000 \times g$  for 5 min) ,Hb concentration was determined by Sahli's (acid haematin) method and neutrophils and lymphocytes were measured by the manual standard procedures, as leukocyte counts were done on Geimsa-stained blood slides with cross-sectional technique (Jain, 1993).

## RESULTS

### Hematological factors

The observed counts of RBCs and PCV level,Hb ,WBCs, lymphocyte, and neutrophil on different weeks of sampling are shown in Tables 1 to 6.

### Mean effect of lead acetate and thiamin on erythrocyte count

Data obtained from The main effect of lead acetate and vitamin B1 on RBCs count of rabbit ' blood in each group on different weeks are shown in Table 1. The results show that no significant differences ( $P > 0.05$ ) were observed in the RBCs of the three groups at the beginning of the study (first week ), but there was a significant increase in group G1 on the 3<sup>rd</sup> and 6<sup>th</sup> week ( $P < 0.05$ ), while the group G3 was a significant decrease on the 3<sup>rd</sup> and 6<sup>th</sup> weeks in comparison with the basal level (1<sup>st</sup> week) ( $P < 0.05$ ) also on the 6<sup>th</sup> week in comparison between groups . In group G2 no significant differences ( $P > 0.05$ ) were observed in RBCs count in all of sampling times in comparison to basal level (first week).

### Hemoglobin concentration

The results regarding hemoglobin concentration showed the following aspects in the table 2: mean values for Hb concentration for all groups were no significant differences ( $P > 0.05$ ) at the sampling in 1<sup>st</sup> week but the group G3 record significant decrease ( $P < 0.05$ ) in 3<sup>rd</sup> and 6<sup>th</sup> week as compared with other groups , also with the basal level significantly.

### The PCV% Counts

the effect of lead acetate and vitamin B1 on PCV level In all the groups, shown in table 3: There were no significant differences ( $P > 0.05$ ) in the PCV level at the sampling in 1<sup>st</sup> and 3<sup>rd</sup> week but the group G3 record significant decrease ( $P < 0.05$ ) in 6<sup>th</sup> week as compared with other groups , also with the basal level (1<sup>st</sup> week).

Table 4 shows the levels of leukocyte were dissimilar in all treated groups on and no significant different between groups in all period except on 3<sup>rd</sup> and 6<sup>th</sup> weeks , the G3 group record significant increased ( $P < 0.05$ ) as compared with other groups ,as well as in continue experiment the total WBCs count in group (G3) were gradually and clearly elevated significantly on

3<sup>rd</sup> and 6<sup>th</sup> week of experiment as in comparison with experiment start of experiment sampling .

### Lymphocyte number

Table 5 shows, the lymphocytes number in group G3 decreased significantly ( $P<0.05$ ) from the 1<sup>st</sup> to 6<sup>th</sup> week. While, in group G2 also decreased from 1<sup>st</sup> to 6<sup>th</sup> week in the mean value, but in 6<sup>th</sup> week increased significantly ( $P<0.05$ ) as compared with the 3<sup>rd</sup> week . In group G1, no significant difference ( $P>0.05$ ) was observed in lymphocyte counts on the 3<sup>rd</sup> to 6<sup>th</sup> week in comparison with 1<sup>st</sup> week.

In comparing the means total of the three groups, significant differences not observed in the lymphocyte counts on 1<sup>st</sup> week ( $P>0.05$ ). while, The

significant decrease ( $P<0.05$ ) in the lymphocyte counts of group G3 on the 3<sup>rd</sup> and 6<sup>th</sup> week in comparison with those in other groups. Also, G1 was significant difference in comparison with G2.

Table 6 shows, the neutrophils number in the group G1 increased from 1<sup>st</sup> to 6<sup>th</sup> weeks , and in group G2 increased from 1<sup>st</sup> to 3<sup>rd</sup> week and afterward decreased significantly ( $P<0.05$ ) , regarded the neutrophils number in group G3 showed that there was a highly increased ( $P<0.05$ ) in the neutrophil counts percent on the 3<sup>rd</sup> and 6<sup>th</sup> weeks in comparison with 1<sup>st</sup> week, In comparing the means of the all of the groups neutrophils number , the significant difference was observed in G3 for the neutrophil numbers on 3<sup>rd</sup> and 6<sup>th</sup> weeks ( $P>0.05$ ).

**Table 1. Effect of lead acetate and vitamin B1 on RBC( $\times 10^{12}$ /L) during varies weeks (means $\pm$ SD)**

Group//period	1 <sup>st</sup> week	3 <sup>rd</sup> week	6 <sup>th</sup> week
G1control group	B4.74 $\pm$ 0.23a	AB4.97 $\pm$ 0.16a	A5.40 $\pm$ 0.21a
G2	A 4.96 $\pm$ 0.28a	A 5.11 $\pm$ 0.24a	A 5.29 $\pm$ 0.17a
G3	A 5.06 $\pm$ 0.02a	AB4.71 $\pm$ 0.13a	B4.30 $\pm$ 0.13b
LSD	0.56		

Means with different small letter in the same column significantly different ( $P<0.05$ )

Means with different capital letter in the same row significantly different ( $P<0.05$ )

**Table 2. Effect of lead acetate and vitamin B1 on Hb(gr/100ml) during varies weeks (means $\pm$ SD)**

Group//period	1 <sup>st</sup> week	3 <sup>rd</sup> week	6 <sup>th</sup> week
G1control group	A10.80 $\pm$ 0.29a	A11.17 $\pm$ 0.12ab	A11.67 $\pm$ 0.12a
G2	A10.62 $\pm$ 0.26a	A11.55 $\pm$ 0.45a	A11.17 $\pm$ 0.78a
G3	A10.95 $\pm$ 0.13a	AB10.45 $\pm$ 0.10b	B9.92 $\pm$ 0.20b
LSD	1.01		

Means with different small letter in the same column significantly different ( $P<0.05$ )

Means with different capital letter in the same row significantly different ( $P<0.05$ )

**Table 3. Effect of lead acetate and vitamin B1 on PCV(%) during varies weeks (means $\pm$ SD)**

Group//period	1 <sup>st</sup> week	3 <sup>rd</sup> week	6 <sup>th</sup> week
G1control group	A35.47 $\pm$ 1.65a	A 34.69 $\pm$ 1.52a	A37.48 $\pm$ 1.14a
G2	A35.17 $\pm$ 1.53a	A35.94 $\pm$ 1.34a	A36.66 $\pm$ 1.16a
G3	A35.94 $\pm$ 0.52a	A34.85 $\pm$ 0.54a	B30.24 $\pm$ 0.78b
LSD	3.49		

Means with different small letter in the same column significantly different ( $P<0.05$ )

Means with different capital letter in the same row significantly different ( $P<0.05$ )

**Table 4. Effect of lead acetate and vitamin B1 on WBC( $\times 10^9$ ) during varies weeks (means $\pm$ SD)**

Group//period	1 <sup>st</sup> week	3 <sup>rd</sup> week	6 <sup>th</sup> week
G1control group	A 5.24 $\pm$ 0.18a	A 5.37 $\pm$ 0.34b	A 5.63 $\pm$ 0.56b
G2	A 5.87 $\pm$ 0.34a	A 6.70 $\pm$ 0.74b	A 5.71 $\pm$ 0.58b
G3	B 6.04 $\pm$ 0.22a	A 9.38 $\pm$ 0.98a	A 8.95 $\pm$ 1.45a
LSD	2.08		

Means with different small letter in the same column significantly different ( $P<0.05$ )

Means with different capital letter in the same row significantly different ( $P<0.05$ )

**Table 5. Effect of lead acetate and vitamin B1 on (lymphocyte %) during varies weeks (means±SD) rabbit**

Group/period	1 <sup>st</sup> week	3 <sup>rd</sup> week	6 <sup>th</sup> week
G1control group	A 63.80±0.038a	B62.84±0.035a	B 63.50±0.018a
G2	A 63.82±0.027a	C60.50±0.038b	B 62.75±0.021b
G3	A 64.45±0.178a	B 59.37±0.026c	C 55.26±0.047c
LSD	0.67		

Means with different small letter in the same column significantly different (P<0.05)

Means with different capital letter in the same row significantly different (P<0.05)

**Table 6. Effect of lead acetate and vitamin B1 on (Neutrophils %) during varies weeks (means±SD)**

Group/period	1 <sup>st</sup> week	3 <sup>rd</sup> week	6 <sup>th</sup> week
G1control group	B 55.31±0.032a	B55.62 ±0.045c	A 58.19 ±0.02b
G2	C 54.43±0.013b	A 57.22 ±0.047b	B 55.56 ±0.033c
G3	C52.27 ±0.049c	B 62.68 ±0.031a	A 74.93 ±0.027a
LSD	0.44		

Means with different small letter in the same column significantly different (P<0.05)

Means with different capital letter in the same row significantly different (P<0.05)

## DISCUSSION

A causative agent of the environmental pollutant is toxic heavy metal (lead), which had effects direct or indirect on the biological, biochemical systems and somatic cells. In addition to, the large amount of internal resources on the environment, so that toxicity remained as a consequential public health problem (El-Mehi and Amin, 2012). Although, the toxicity mechanism for lead was no well defined, despite the studies showed the toxic effects of lead occur as a consequence of its propensity for disrupting the delicate pro oxidant/antioxidant balance, which is found within cells (Donaldson and Knowles, 1993; Monteiro *et al.*, 1986).

In this study, the Erythrocytes count in group G2 (lead acetate) was in a less level till the 6<sup>th</sup> week, but this number in group G3 (thiamine together with lead acetate) showed less effect and increase in a slightly in the 3<sup>rd</sup> and 6<sup>th</sup> week. Hence, in group G1 the increase of RBCS value has been done with more delay.

In this study the prominent finding is that the presence of vitamin B1 with lead acetate diminished its injurious effects on the RBCs count. In agreement with our findings, (Newairy and Abdou, 2009; Aleksandra *et al.*, 2014), indicated that the effect of lead acetate induced oxidative damage in the cellular membranes, lead to destabilized and this effect cause decreases fluidity of the cell membrane and then increase the amount of erythrocyte hemolysis. (Lawton and Donaldson, 1991) referred to hemolysis accrue in the final result of membrane lipid peroxidation and ROS production in RBC). On the other hand, that the role of lead in anemia production due to RBCs destruction has been mentioned by (Patrick, 2006), hence that in this study the effect has been significant when studying the hematological profiles, the number of RBC count in the

group getting lead acetate have been less than group G3 (thiamine together with lead acetate) and the control group, this results possible protective *role of thiamine* on the cells membranes through avoid cellular protein damage and reduce oxidative stress on the cells membranes (Aleksandra, *et al.*, 2014). the decrease hemoglobin concentration was significantly in group G2 due to effect of lead acetate on enzyme (aminolevulinic acid Dehydrates) responsible on serious generation of hemoglobin, while Hb concentration of the group fed thiamine within lead acetate (G3) have been no effect also the control group. that due to effect of thiamine on increased Hb concentration by direct action of thiamine on increase enzymes activity, and some elements digestibility of copper and hem, it was important in the hemoglobin syntheses, moreover effect on the antioxidant enzymatic defense system (Flora, 1986; Chunhong, 2007).

But PCV counts showed not statistically significant in all periods except (6<sup>th</sup> week), group G2 was recorded significant decrease, this result due to the direct effect of lead on the source of erythrocyte forming (bone marrow) lead to decrease the erythroid progenitor cells and reduced mitotic and redouble ability (Stec, 2003). On the other hand, the (Sakata *et al.*, 2007), in study mention that inversely proportion of concentration erythropoietin hormone, consider important growth factor and responsible for RBCs syntheses and regulation. Hence, body's first barrier activity of defensive are leucocytes, the total number of white blood cells were decreased due to the treatment with lead acetate, represented by the significant rise in both the total number for the white blood cells and the percentage of the neutrophil cells, while the percentage of lymphocytes decreased, This confirmed the findings of other studies that lead exposure

causes an increase in the total number of WBCs (Mugahi, *et al.*, 2003) in lab. animals. Which partly due to the high number of neutrophil cells, especially since these cells are functionally mature, and fastest response as compared with rest of the cells (DiLorenzo, *et al.*, 2006). Whereas, the polynuclear cells specifically neutrophils, can be affected by this heavy metals. The development process of various types of WBCs influence by lead acetate, through disrupt postnatal development and then lead to a sudden deterioration of neutrophilic and abnormal neutrophils (Sharma *et al.*, 2012). When neutrophils are faced with foreign body, activity of the NADPH oxidase enzyme on the cell surface membrane increases due to released very high oxygen than the normal. Therefore, the result of severely destructive when the interactions in neutrophils and cause neutrophil membrane desolation (Hodgson *et al.*, 2006). Changes in the neutrophils and lymphocytes number that are type of body immunity system response, (Rahmat *et al.*, 2015). Chandra referred to the individual differences that observe within some the species. In this study found that has been earned about the effect of the lead on blood cells, was increasing the number of the neutrophil cells and its keep up a high level for a longer time (6<sup>th</sup> week) in the group G2, which is characterized as a neutrophilia. that agreement with (Di Lorenzo *et al.*, 2006), whom reported that increasing neutrophil counts when exposed to lead. Concerning the lymphocyte count, the administration lead acetate caused a significant decrease in lymphocyte count while, Treatment of rabbits with thiamine at a dose of 100 mg/head every 48h, to some extent improved the lead induced changes in neutrophil and lymphocyte counts in

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the group G2. Thus it appears that thiamine has the ability to protect by reduced effects of lead acetate on the total count or differential count of immune cells. Some researchers mentioned that possibly thiamine to avoid destroyed, and maintenance of cells membrane by the formation of a lead-thiamine metabolic complex inhibit the absorption of lead in tissues or The efficacy of the thiamin to remove metals from the biological system depends on their ability to form stable complexes with the toxic metal ions and to enhance their excretion from the body without affecting the levels of essential trace elements, and also thiamin ability to contact with phospholipids layers in cells membrane lead to integration the membranes and support cells actions (Valko *et al.*, 2006; Valko *et al.*, 2007).

## CONCLUSION

According to above investigation, its deduced that lead acetate can be important agents in destruction of RBCs and effective on increase the counts of neutrophils (neutrophilia), and decline lymphocytes. The combination of vitamin B1 with lead acetate reduced toxicity damage of lead acetate in the blood tissues. Also can be expressed that added vitamin B1, officiates to counteract the unhealthy effects of the environmental exposure to lead.

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## CONFLICT OF INTEREST

No interest



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