



## Protective effects of alpha-lipoic acid against lead-induced oxidative stress in erythrocytes of rats

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### ABSTRACT

The present study was carried to evaluate the protective effects of alpha-lipoic acid against lead (Pb) induced oxidative stress to erythrocytes in rats. Eighty male albino rats were divided into four groups containing 20 rats each. Group I (control): administered distilled water. Group II (Lead exposed group): received lead acetate (30 mg/kg body weight of 1/20th of LD50) orally and once per day over a period of 10 weeks. Group III (Lead+ Alpha-lipoic acid treated group): received lead acetate (30 mg/kg body weight) and treated daily with alpha-lipoic acid (54 mg/kg body weight/ i.p). Group IV(alpha-lipoic acid treated normal group): 54 mg/kg body weight/ i.p. Serum used for determination of nitric oxide (NO), urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-1B (IL-1B) and moreover, erythrocyte hemolysate were processed for the determination of L-Malondialdehyde (L-MDA), catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPx) and reduced Glutathione (GSH). The obtained results revealed that, a significant increase in serum urea, creatinine and ALT, AST and (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-1B (IL-1B) concentrations and hemolysate L-MDA level were observed in lead intoxicated rats. However, administration of alpha-lipoic acid in lead intoxicated rats exhibited a significant decreased in all mentioned parameters. On the other hand, a significant decreased in erythrocyte CAT, SOD and GPx activities, and GSH concentration were observed in lead intoxicated rats. Meanwhile, alpha-lipoic acid administrations in lead intoxicated rats resulted in significant increase in all mentioned parameters. It could be concluded that, the potential of alpha-lipoic acid as a powerful agents and may be useful as an antioxidants in combating free radical-induced oxidative stress and tissue injury that is a result of lead toxicity. Also, these compounds could be also applicable as a cyto-protective against oxidative stress of tissue damage mediated by heavy metals intoxication.

**Keywords:** Antioxidant enzymes, lead, oxidative stress, alpha-lipoic acid, cytokines

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### 1. INTRODUCTION

Lead (Pb), one of the oldest known metals, is a pervasive and persistent environmental occupational toxic metal, and Pb poisoning remains a health threat (Zbakh and Abbassi, 2012). It is a dangerous heavy metal and harmful even in small amounts. Nevertheless, humans get exposed to Pb through their environment and diet so that, more than 75% of lead-exposure for the

general population comes from ingestion (Patrick, 2006). Lead absorption by ingestion depends on factors such as the particle size, physical form, gastrointestinal transit time and nutritional status of a person. Lead absorption increases, with increasing age, making children and infants more vulnerable to lead intoxication (Campbell et al., 2004). The manifestations of Pb poisoning in humans are nonspecific. They may include weight loss, anemia, memory

loss, nephropathy, infertility, liver, testis and heart damages (Gurer-Orhan et al., 2004).

Lead-induced oxidative stress in blood, corpus cell and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Waters et al., 2008). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasani et al., 2001). Also, induced kidney injury was related to the increase production of reactive oxygen species (ROS), and to induce oxidative stress, excite toxicity, DNA damage and apoptosis (Dai et al., 2013).

Antioxidants are substances, inhibit or delay oxidation of a substrate while present in minute amounts. They easily oxidized by ROS in a biological system, decreasing the rate at which the ROS react with cellular components like lipid membranes, DNA, or proteins. The most important source of antioxidants is provided by nutrition (Flora, 2002).

Alpha-lipoic acid is water and lipid soluble, a property that allows it to concentrate in cellular and extracellular environments. Exogenous LA is rapidly absorbed from the diet, and is reduced inside the cell to dihydrolipoic acid (DHLA), the most active form of the substance (May et al., 2007). Alpha-Lipoic Acid (LA) is a naturally occurring compound which functions as a cofactor in several mitochondrial multi-enzyme complexes involved in energy production in humans and animals (Shay et al., 2009). LA acts as coenzyme of pyruvate and the alpha-ketoglutarate dehydrogenase multienzyme complex of the tricarboxylic acid cycle and has metal chelating, free radical scavenging and antioxidant-regenerating abilities (Caylak et al., 2008). It protects against oxidative stress both in peripheral tissues and central nervous system (Winiarska et al., 2008).

Accordingly, the present study was designed to investigate the beneficial effects of alpha-lipoic acid on biomarkers of oxidative stress and antioxidant enzymatic states in erythrocyte in addition to changes of serum pro-inflammatory cytokines in lead intoxicated rats.

## 2. MATERIALS AND METHODS

### 2.1. Experimental animals:

One hundred white male albino rats of 8-10 weeks old and weighing 160 – 200 gm were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad-libitum. All rats were acclimatized for minimum period of two weeks prior to the beginning of study.

### 2.2. Chemicals and drugs

All chemicals were of analytical grade and obtained from standard commercial suppliers. The drug and chemicals used in the present study were:

*Lead-acetate:* Lead-acetate has molecular weight 379.33. Each one gram of Lead-acetate 72% contains 521 mg of Lead-acetate. It was manufactured by Riedel-Dehoen Ag Seelze- Hannover, West Germany. Lead-acetate was dissolved in distilled water, freshly prepared and administered orally and daily at a dose level of 30 mg/kg body weight (1/20 of L.D.50).

*Alpha- Lipoic acid (Thiotacid)<sup>R</sup>:* Thiotacid was obtained as pack of five ampoules of 10ml solution. Each ampoule contains thioctic acid (alpha lipoic acid) 300 mg. Alpha-lipoic acid (Thioctic acid)<sup>®</sup> manufactured by EVA pharma for pharmaceuticals and Medical Appliances, Egypt. Alpha lipoic acid was injected intraperitoneal in a daily dose of 54 mg/kg body weight (Gruzman et al., 2004).

### 2.3. Experimental design

After acclimatization to the laboratory conditions, the animals were randomly divided into five groups (twenty rats each) placed in individual cages and classified as follow:

Group I (control normal group): Rats received no drugs, served as control non-treated for all experimental groups.

Group II (Lead-acetate exposed group): Rats received cadmium chloride 1/20 of LD<sub>50</sub> (30 mg/kg body weight) orally and once per day over a period of 10 weeks.

Group III (Lead-acetate + Alpha-lipoic acid treated group): Rats received Lead-acetate (30 mg/kg body weight) and treated daily with alpha-lipoic acid (54 mg/kg body weight/ i.p).

Group IV (Alpha-lipoic acid treated normal group): Rats treated daily with alpha-lipoic acid (54 mg/kg body weight/ i.p).

### 2.4. Sampling:

#### 2.4.1. Blood samples:

Blood samples were collected by ocular vein puncture from all animal groups 2 times along the duration of experiment in dry, clean and screw capped heparinized tubes and plasma were separated by centrifugation at 2500 r.p.m for 15 minutes. After plasma separation, erythrocytes were washed 3 times with an equal volume of cold saline. 1mlRBC lyses with 4 ml distilled water in dry sterile capped tubes for subsequent biochemical analysis.

The rest of blood samples were collected in dry, clean test tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was separated by automatic pipette and received in dry sterile tubes, processed directly for ALT and AST lipoic acid to lead intoxicated rats caused significant increase in serum NO with

determination, then kept on deepfreeze at -20 until use for subsequent biochemical analysis. All sera were analyzed for the following parameters: NO, Creatinine, Urea, TNF- $\alpha$ , IL-6 and IL-1b.

### 2.5. Biochemical analysis

Serum NO, Creatinine, Urea, ALT and AST, TNF- $\alpha$ , (IL-6) and (IL-1B) were determined according to the method described by Vodovotz, (1996), Tietz (1986), Tietz (1990), Schumann et al., (2002), Beyaert and Fiers (1998), Chan and Perlstein (1987), and Rat IL-1 beta ELISA (RayBiotech, Inc Company, Cat#: ELR-IL1b), respectively. Moreover, erythrocyte MDA, CAT, SOD, GPx and GSH were determined according to the method described by Esterbauer et al., (1982), Sinha, (1972), Glazer (1990), Gross et al., (1967), Necheles et al., 1968) and Beutler et al.,(1963) respectively.

### 2.6. Statistical Analysis

The results were expressed as mean  $\pm$  SE and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when  $p < 0.05$ .

## 3. RESULTS

The obtained data in table (1) revealed that, lead intoxicated rats at (eight) weeks ,showed significant decrease in serum NO and hemolysate SOD, CAT and GSH with non-significant decrease in GPx accompanied with significant increase hemolysate MDA and serum urea , creatinine, ALT, AST , IL-6, IL-1B and TNF- $\alpha$  concentration when compared with normal control group. Treatment with alpha-

significant decrease in hemolysate MDA and serum urea , creatinine , ALT, AST , IL-

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6,IL-1B and TNF- $\alpha$  concentration when compared with lead intoxicated group.

The obtained data in table (2) revealed that, lead intoxicated rats at (ten) weeks ,showed significant decrease in serum NO and hemolysate CAT, GPx and GSH with non-significant decrease in SOD accompanied with significant increase in hemolysate MDA and serum urea , creatinine, ALT,

AST , IL-6, IL-1 $\beta$  and TNF- $\alpha$  concentration when compared with normal control group. Treatment with alpha-lipoic acid to lead intoxicated rats caused significant increase in serum NO with significant decrease in hemolysate MDA and serum urea, creatinine, ALT, AST, IL-6, IL-1B and TNF- $\alpha$  concentration when compared with lead intoxicated group.

Table (1) Protective effect of alpha- lipoic acid on some biochemical parameters in lead intoxicated rats and their control (eight weeks).

Parameters	Group I	Group II	Group III	Group IV
NO (mmol/L)	69.30 $\pm$ 3.65 <sup>a</sup>	47.44 $\pm$ 2.25 <sup>b</sup>	18.65 $\pm$ 2.18 <sup>c</sup>	67.70 $\pm$ 4.24 <sup>a</sup>
ALT(U/L)	41.93 $\pm$ 5.92 <sup>c</sup>	114.47 $\pm$ 1 7.20 <sup>b</sup>	186.86 $\pm$ 7.76 <sup>a</sup>	44.97 $\pm$ 5.45 <sup>c</sup>
AST(U/L)	61.72 $\pm$ 4.79 <sup>c</sup>	170.76 $\pm$ 4.36 <sup>b</sup>	264.05 $\pm$ 9.91 <sup>a</sup>	59.59 $\pm$ 3.56 <sup>c</sup>
Urea(mg/dl)	25.66 $\pm$ 2.82 <sup>bc</sup>	29.35 $\pm$ 3.02 <sup>b</sup>	52.16 $\pm$ 3.04 <sup>a</sup>	23.59 $\pm$ 3.37 <sup>b</sup>
Creatinine(mg/dl)	1.340 $\pm$ .268 <sup>a</sup>	0.670 $\pm$ .023 <sup>b</sup>	0.540 $\pm$ .068 <sup>b</sup>	0.460 $\pm$ .060 <sup>b</sup>
IL- 6 (pg/ml)	56.76 $\pm$ 9.91 <sup>b</sup>	158.42 $\pm$ 15.15 <sup>a</sup>	67.85 $\pm$ 21.85 <sup>b</sup>	45.73 $\pm$ 9.91 <sup>b</sup>
IL- 1 $\beta$ (pg/ml)	254.16 $\pm$ 55.45 <sup>b</sup>	831.98 $\pm$ 48.49 <sup>a</sup>	333.82 $\pm$ 65.25 <sup>b</sup>	194.04 $\pm$ 71.24 <sup>b</sup>
TNF- $\alpha$ (pg/ml)	34.07 $\pm$ 6.91 <sup>b</sup>	74.04 $\pm$ 5.01 <sup>a</sup>	40.62 $\pm$ 4.27 <sup>b</sup>	23.68 $\pm$ 5.44 <sup>b</sup>
MDA (mmol/L)	71.32 $\pm$ 14.06 <sup>b</sup>	137.42 $\pm$ 11.40 <sup>a</sup>	74.08 $\pm$ 8.98 <sup>b</sup>	45.04 $\pm$ 10.86 <sup>b</sup>
SOD(U/L)	24.14 $\pm$ 6.29 <sup>ab</sup>	7.31 $\pm$ 1.99 <sup>b</sup>	26.92 $\pm$ 6.56 <sup>a</sup>	32.20 $\pm$ 6.09 <sup>a</sup>
CAT(mmol/L)	56.41 $\pm$ 4.61 <sup>ab</sup>	19.92 $\pm$ 3.94 <sup>c</sup>	45.38 $\pm$ 3.39 <sup>b</sup>	63.83 $\pm$ 6.86 <sup>a</sup>
GPx(ng/ml)	33.55 $\pm$ .599 <sup>a</sup>	15.68 $\pm$ 2.39 <sup>c</sup>	24.32 $\pm$ 1.27 <sup>b</sup>	34.96 $\pm$ 3 <sup>a</sup>
GSH(ng/ml)	9.56 $\pm$ 1.13 <sup>a</sup>	0.431 $\pm$ .089 <sup>c</sup>	5.45 $\pm$ .604 <sup>b</sup>	7.96 $\pm$ 1.06 <sup>ab</sup>

Data are presented as (Mean  $\pm$  S.E) S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ( $P \leq 0.05$ ).

Table (2) Protective effect of alpha- lipoic acid on some biochemical parameters in lead intoxicated rats and their control (ten weeks).

Parameters	Group I	Group II	Group III	Group IV
NO (mmol/L)	51.68 $\pm$ 2.71 <sup>a</sup>	19.70 $\pm$ 2.77 <sup>b</sup>	52.20 $\pm$ 4.30 <sup>a</sup>	64 $\pm$ 3.25 <sup>a</sup>
ALT(U/L)	55.79 $\pm$ 8.02 <sup>b</sup>	213.55 $\pm$ 21.40 <sup>a</sup>	76.27 $\pm$ 16.45 <sup>b</sup>	54.84 $\pm$ 5.86 <sup>b</sup>
AST(U/L)	65.92 $\pm$ 4.74 <sup>c</sup>	263.42 $\pm$ 11.06 <sup>a</sup>	264.05 $\pm$ 9.91 <sup>a</sup>	59.58 $\pm$ 6.79 <sup>c</sup>
Urea(mg/dl)	56.37 $\pm$ 5.94 <sup>a</sup>	27.09 $\pm$ 3.91 <sup>b</sup>	27.82 $\pm$ 2.72 <sup>b</sup>	59.58 $\pm$ 6.79 <sup>c</sup>
Creatinine(mg/dl)	.440 $\pm$ .046 <sup>b</sup>	.583 $\pm$ .041 <sup>b</sup>	.970 $\pm$ .145 <sup>a</sup>	.583 $\pm$ .081 <sup>b</sup>
IL- 6 (pg/ml)	48.60 $\pm$ 14.42 <sup>b</sup>	63.67 $\pm$ 7.46 <sup>b</sup>	157.90 $\pm$ 10.68 <sup>a</sup>	48.05 $\pm$ 8.42 <sup>b</sup>
IL- 1 $\beta$ (pg/ml)	147.82 $\pm$ 15.05 <sup>c</sup>	465.50 $\pm$ 35.03 <sup>b</sup>	761.12 $\pm$ 85.38 <sup>a</sup>	210.64 $\pm$ 53.49 <sup>c</sup>
TNF- $\alpha$ (pg/ml)	19.03 $\pm$ 2.80 <sup>c</sup>	48.68 $\pm$ 3.13 <sup>b</sup>	93 $\pm$ 7.24 <sup>a</sup>	19.30 $\pm$ 4.37 <sup>c</sup>
MDA (mmol/L)	29.72 $\pm$ 1.94 <sup>b</sup>	64.81 $\pm$ 8.61 <sup>b</sup>	121.29 $\pm$ 20.85 <sup>a</sup>	54.27 $\pm$ 6.54 <sup>b</sup>
SOD(U/L)	28.14 $\pm$ 2.75 <sup>a</sup>	17.29 $\pm$ 1.09 <sup>b</sup>	4.74 $\pm$ 1.53 <sup>c</sup>	23.41 $\pm$ 4.02 <sup>a</sup> <sup>b</sup>
CAT(mmol/L)	63.36 $\pm$ 2.23 <sup>a</sup>	52.82 $\pm$ 2.62 <sup>a</sup>	28.28 $\pm$ 6.45 <sup>b</sup>	56.53 $\pm$ 7.28 <sup>a</sup>
GPx(ng/ml)	35.80 $\pm$ 3.13 <sup>a</sup>	20.25 $\pm$ .795 <sup>b</sup>	13.89 $\pm$ 1.63 <sup>b</sup>	33.75 $\pm$ 4.18 <sup>a</sup>
GSH(ng/ml)	7.85 $\pm$ .852 <sup>a</sup>	6.63 $\pm$ 1.43 <sup>a</sup>	.441 $\pm$ .162 <sup>b</sup>	7.23 $\pm$ 1.59 <sup>a</sup>

Data are presented as (Mean  $\pm$  S.E) S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ( $P \leq 0.05$ ).

#### 4. DISCUSSION

Lead intoxicated rats showed significant decrease in serum nitric oxide concentration with normal control group. Lead treatment was associated with increased ROS production and inactivation of NO. Thus, high ROS levels after lead-exposure may increase the presence of superoxide anion, raising the probabilities of an interaction between NO and ROS to produce a peroxy nitrite, highly deleterious molecule (Robles *et al.*, 2007). Treatment with alpha-lipoic acid to lead intoxicated rats caused significant increase serum nitric oxide concentration when compared with lead intoxicated group.

In present study a significant increase in serum ALT and AST in lead acetate treated rats all over the periods of the experiment when compared with normal control group. These results came in accordance with the recorded data of Abdel Aal and Abeer, (2008) they reported that Lead administration resulted in increase serum alkaline phosphatase (ALP), ALT and AST. This is because lead is known to produce oxidative damage in the liver tissues by producing peroxidation of membrane lipids and cause derangement of several hepatic biochemical pathways and energy metabolism (Taki *et al.*, 1985).

Treatment with Alpha-lipoic acid to lead intoxicated male rats caused a significant decrease in elevated serum (ALT), (AST) activities was observed in lead intoxicated male rats after ten weeks of the experiment. These results came in accordance with the recorded data of Osfor *et al.*, (2010), who reported that, Alpha lipoic acid (ALA) diminished ALT, AST in lead intoxicated rats.

A significant increase in serum urea and creatinine concentration was observed in lead intoxicated male rats all over the periods of the experiment when compared with normal control group. These results are nearly similar to those reported by Jurczuk *et al.*, (2007) reported that, increased level of serum urea, uric acid and creatinine in Pb treated rats. The elevation in the serum of creatinine and urea caused by lead suggest that renal function impairment which might result from intrinsic renal lesions, decreased perfusion of the kidney obstruction of lower urinary tract or due to deranged metabolic process caused by this metal (Cameron and Greger, 1998).

Treatment with Alpha-lipoic acid to lead intoxicated male rats caused a significant decrease in serum urea and creatinine activity all-over the periods of the experiment, when compared with lead exposed group. These results came in accordance with the recorded data of Osfor *et al.*, (2010) reported that, Alpha-lipoic acid decrease urea and creatinine levels in lead intoxicated rats. Similarly, El-Beshbishy *et al.*, (2011) showed that, Alpha-lipoic acid intake declined Serum creatinine and urea levels compared to control. Burke *et al.*, (2003) reported that; Alpha lipoic acid supplementation has been shown to enhance the uptake of creatinine within muscle cells. It protects kidney tissues by inhibiting neutrophil infiltration, balancing the oxidant-anti-oxidant status, and regulating the generation of inflammatory mediators (Shanmugarajan *et al.*, 2008)

The obtained results revealed that, a significant increase in (TNF- $\alpha$ ) concentration, (IL-6) and (IL-1B) activity, was observed in lead intoxicated male rats all over the periods of the experiment when compared with normal control group. Similarly, Bah *et al.*, (2011) who reported that, Increase TNF- $\alpha$  in Pb-exposed rats,

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levels of TNF- $\alpha$  significantly increasing in a gradual manner with increasing doses of Pb (Kumawat et al., 2014). The stimulatory effect of Pb appears to be due to a modification of the expression of membrane TNF- $\alpha$  receptors, which are modulated by Pb. Lead intake resulted in significant increases interleukin-6 (IL-6), this cytokines were assayed as indicators of inflammation and tissue damage in heart degenerative cells and serum of rats treated with lead (Marwa and Hassanein, 2013).

Treatment with Alpha-lipoic acid to lead intoxicated male rats, significantly reduced elevated (TNF- $\alpha$ ), (IL-6) and (IL-1B) concentration in lead intoxicated male rats all over the periods of the experiment. These results came in accordance with the recorded data of Park et al., (2005) who reported that, ALA reduces the serum levels of pro-inflammatory cytokines such as (TNF- $\alpha$ ), (IL-6) and (IL-1B). Sola et al., (2005) who recorded that, treatment with lipoic acid was associated with significant reductions in plasma levels of pro-inflammatory mediators, such as (IL-6), suggesting that alpha-lipoic acid may improve endothelial dysfunction via anti-inflammatory and antithrombotic mechanisms.

Lead cause oxidative damage in various peripheral organs by enhancing lipid peroxidation (Hamadouche et al., 2009). The oxidation is usually caused by ROS like oxyl radicals, peroxy radicals and hydroxyl radicals (Hsu and Guo, 2002). The obtained results revealed that, lead intoxicated rats showed significant increase in erythrocytes L-MDA concentration when compared with normal control group. Likewise, Omobowale et al., (2014) observed that, Treatment with lead gave rise to significant increase in MDA and H<sub>2</sub>O<sub>2</sub> generation both in the liver and the erythrocytes following exposure to lead. Also, Ponce-Canchihuamán et al., (2010) observed that, administered 25

mg/0.5 ml of lead acetate intraperitoneally to rats' weekly, level of MDA was significantly increased with respect to the control. And this could be due to lead induced inhibition of radical scavenging enzymes like GST and SOD (Haleagrahara et al., 2011).

Treatment with Alpha-lipoic acid to lead intoxicated male rats significantly reduced elevated erythrocyte Lipid peroxidation (L-MDA) concentration in lead intoxicated male rats all over the periods of the experiment. These results came in accordance with the recorded data of, Abdel Aal and Abeer, (2008) ALA is capable of diminish lipid peroxidation which was determined by estimating serum MDA level in lead treated group. Accordingly, Akpınar et al., (2007) demonstrated that, rats given alpha lipoic acid while being exposed to stress were protected from lipid peroxidation by the antioxidant properties of alpha lipoic acid. The protective action of alpha lipoic acid against lipid peroxidation as a factor modifying membrane organization may due to alpha lipoic acid's ability to scavenge the free radicals, which are produced during the peroxidation of lipids.

Lead intoxicated rats showed significant decrease in erythrocytes GSH concentrations. These results came in accordance with the recorded data of Al abbassi et al., (2008) reported that, Daily treatment of rats with lead acetate significantly reduces GSH levels in RBCs, brain, liver and kidney compared normal. These results are nearly similar to those reported by Omobowale et al., (2014) .who reported that, exposure of GSH is a tripeptide containing cysteine with a reactive SH group and reductive potency. Lead binds exclusively to the -SH group, which decreases the GSH levels and can interfere with the antioxidant activity of GSH (Bechara, 2004).

Treatment with Alpha-lipoic acid to lead intoxicated male rats, significantly increase erythrocyte (GSH) concentration was observed in lead intoxicated male rats all over the periods of the of the experiment. These results came in accordance with the recorded data of Gurer *et al.*, (1999) recorded that, LA in relieving Lead-induced oxidative stress includes the increase in GSH content in animals after LA administration. Also, Pande, and Flora, (2002) reported that, administrations of LA increase GSH level towards normal on blood and soft tissue of lead ,also suggest beneficial role of LA during chelation therapy of lead intoxication.

Elevated GSH (kidney, brain, RBC) levels after LA administration to CHO cells and Fischer 344 rats challenged by oxidative stress via lead treatment. DHLA is known to reduce glutathione disulfide (GSSG) to GSH; however, elevations in GSH cannot be solely explained by reduction of GSSG, because GSSG is normally present at less than 10% of GSH concentrations (Halliwell *et al.*, 1989). They suggested that LA administration can induce increases in GSH levels by facilitating transport of cystine, the limiting factor in GSH synthesis, into the cells. Once LA is taken up by the cell it is immediately reduced to DHLA that is then released. The released DHLA induces a chemical reduction of extracellular cystine to cysteine. Cysteine can be taken up rapidly (10 times more) by the cells than cystine and can then be used in the biosynthesis of GSH.

A significant decrease in erythrocytes catalase (CAT) Superoxide, dismutase (SOD) activity and Glutathione peroxidase (GPX) were observed in lead exposed rats. These results came in accordance with the recorded data of Imran Khan *et al.*, (2008) observed chronic lead exposure of residential and commercial painters shows decreased erythrocyte CAT, SOD, GPx and lipid peroxidation. Also, Omobowale *et al.*,

(2014) recorded that, Activities of (GPx), (GST), (CAT) and (SOD) which are major antioxidant enzymes significantly crashed at both exposure and withdrawal of lead in the liver and the erythrocytes. This is an indication that lead toxicity targets antioxidant enzymes that offer protection against oxidative stress and membrane lipid peroxidation. More so, potential toxicity of lead can therefore be ascribed to its inhibiting effects on the antioxidant defence system. These antioxidant enzymes are the primary enzymatic defense against toxic oxygen reduction metabolites, and each enzyme has an integral function in free radical modulation. Thus, the accumulated free radical could consume SOD, CAT, and GSH-Px in the kidney and liver. Moreover, if the balance between reactive oxygen species (ROS) production and antioxidant defense was disrupted, the enzyme may be exhausted and its concentration may be depleted (Liu *et al.*, 2010).

Many studies have shown that lead can also alter antioxidant activities by inhibiting functional SH groups in several enzymes such as SOD, CAT and GPx, because of its high affinity for sulfhydryl (SH) groups in these enzymes (Hsu and Guo, 2002). Moreover, if the balance between ROS production and antioxidant defenses is broken, the enzyme may be exhausted and its concentration depletions. The activities of antioxidant enzymes in rat kidney, including SOD, CAT and GPx, were dramatically decreased by the treatment of lead.

Treatment with Alpha-lipoic acid to lead intoxicated male rats, significantly increase erythrocyte (CAT) and (SOD) concentration and non significant increase in erythrocyte (GPX) after eight weeks. Meanwhile, after ten weeks a significant increase in erythrocyte (CAT), (SOD) and (GPX). These results came in accordance with the recorded data of El-Beshbishy *et al.*, (2011)

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reported that, Catalase, SOD and GPx levels kidney homogenate increased in treated alpha lipoic acid group. Also, Jesudason et al., (2008) reported that, increased activity of SOD, CAT, GPx and glutathione level were observed in systemic inflammation after alpha lipoic acid treatment.

The obtained results revealed that, a significant increase in Kidney and liver lead residues concentration was observed in lead intoxicated male rats after eight and ten weeks of the experiment. These results came in accordance with the recorded data of Alcaraz-Contreras et al., (2011) reported that, lead exposure caused a significant increase in its levels in blood, brain, liver, kidney, and bone samples compared with samples from controls. The high concentration of lead in different tissues has been associated with oxidative stress, which might be responsible, at least in part, for lead's toxic effects. Also, Aziz et al., (2012) show that, the high increase of lead content was observed in the kidneys and livers of rats. Similarly, Liu et al., (2012) recorded that, the Pb levels in blood and kidney of Pb-treated rats are significantly higher than those of control rats. Lead appears within and among soft tissues where the highest concentration of lead seems to accumulate specially in those organs and tissues with the highest mitochondrial activity also, the function of oxidative damage in Pb- and Cd-induced changes in steroidogenesis in the liver and kidney (Dai et al., 2013).

Treatment with Alpha-lipoic acid to lead intoxicated male rats, significantly reduced elevated Kidney and liver lead residues in lead intoxicated male rats after eight and ten weeks from the onset of treatment with  $\alpha$ -lipoic acid. These results came in accordance with the recorded data of Osfor et al., (2010) reported that, Alpha lipoic acid (ALA) decrease lead levels in serum and kidney tissue compared to the control rats. Treatment with  $\alpha$ -lipoic acid led to

decreased lead burden and lipid peroxide formation. These beneficial effects might be due to the chelation of lead by the thiol chelators and the antioxidant action of  $\alpha$ -lipoic acid. Antioxidant ALA would repair the damaged tissues effectively and improve the thiol status. ALA as a potent antioxidant not only scavenges free radicals, but also raises the intracellular level of antioxidants by recycling them, and chelates heavy metals to prevent free radical generation. ALA antioxidant role involves protecting cells from damage by preventing the destruction of lipids in cell membranes (Pande, and Flora, 2002).

It could be concluded that, the potential of alpha-lipoic acid a powerful agents and may be useful as an antioxidants in combating free radical-induced oxidative stress and tissue injury that is a result of lead toxicity. Also, these antioxidants have a protective antioxidant and anti inflammatory effects and could be also applicable as a cytoprotective against oxidative stress of tissue damage mediated by lead intoxication.

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## التأثيرات الوقائية لحمض ألفالبيويك ضد الإجهاد التأكسدي المحدث بالرصاص لخلايا الدم الحمراء في الفئران.

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### الملخص العربي

في هذه الدراسة تم تقييم التأثير الواقي لحمض ألفا لبيويك على الإنزيمات مانعة التأكسد في خلايا الدم الحمراء في الفئران المسممة بالرصاص المسبب للإجهاد التأكسدي. هذا وقد أستخدم لأجراء هذه الدراسة عدد 80 من ذكور الفئران أعمارهم تتراوح من 12-16 أسبوع و أوزانها من 220-250 جرام وقد قسمت إلي أربعة مجموعات متساوية اشتملت كل مجموعة على عدد عشرون فأر وتم توزيعها كالاتي: المجموعة الأولى: (المجموعة الضابطة): اشتملت على 20 فأر لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (المجموعة المسممة بالرصاص): تكونت من 20 فأر تم تجريعهم للرصاص فقط يوميا عن طريق الفم بجرعة مقدارها 30 ملليجرام لكل كيلوجرام من وزن الجسم لمدة 10 أسابيع. المجموعة الثالثة: (مجموعة الرصاص + الألفالبيويك): اشتملت على 20 فأر تم تجريعهم للرصاص يوميا عن طريق الفم بجرعة مقدارها 30 ملليجرام لكل كيلوجرام من وزن الجسم وحمض ألفا لبيويك يوميا في التجويف البريتوني بجرعة مقدارها 54 ملليجرام لكل كيلوجرام من وزن الجسم طوال فترة التجربة. المجموعة الرابعة: (مجموعة حمض ألفا لبيويك الطبيعية): اشتملت على 20 فأر تم حقنهم بحمض ألفا لبيويك يوميا في التجويف البريتوني بجرعة مقدارها 54 ملليجرام لكل كيلوجرام من وزن الجسم طوال فترة التجربة. وقد تم جمع عينات الدم من الجيب الوريدي خلف العين في أنابيب الهيبارين حيث تم مزجها جيدا لفصل كرات الدم الحمراء المستخدمة في تحضير حاصل الإنحلال الدموي (الهيموليزات) واستخدام مباشرة لقياس تركيز المالونديالدهيد، إنزيم الكتاليز والجلوتاتايون بيروكسيديز ووسوبر أكسيد ديسميوتيز وإنزيم الجلوتاتايون ريديكتاز، وتجميع الجزء الآخر في أنابيب نظيفة، جافة ومعقمة وترك العينة قليلا حتى تتجلط ثم فصل السيرم بواسطة جهاز الطرد المركزي وذلك عند سرعة 2500 لفة في الدقيقة لمدة 15 دقيقة بعدها تم جمع السيرم في أنابيب جافة ومعقمة بواسطة ماصة اتوماتيكية واستخدم هذا السيرم مباشرة لقياس تركيز نيتريك اسيد، اليوريا، الكرياتنين، الألانين ترانسفيريز، اسبارتيت ترانسفيريز، الأنترلوكين6، الأنترلوكين1 - بيتا وعامل التنخر الورمي الفا. وقد أسفرت نتائج التحليل البيوكيميائي فيمجموعة الفئران المحدث بها التسمم بالرصاص عن وجود زيادة في كلا من المالونديالدهيد، اليوريا، الكرياتنين، الألانين ترانسفيريز، اسبارتيت ترانسفيريز، الأنترلوكين6، الأنترلوكين1 - بيتا وعامل التنخر الورمي الفا. وجود انخفاض معنوي في كلا من نيتريك اسيد، إنزيم الكتاليز والجلوتاتايون بيروكسيديز ووسوبر أكسيد ديسميوتيز، إنزيم الكتاليز والجلوتاتايون الممتز في حين ارتفع نيتريك اسيد، إنزيم الكتاليز والجلوتاتايون بيروكسيديز ووسوبر أكسيد ديسميوتيز وإنزيم الجلوتاتايون الممتز. ولخصت الدراسة أن حمض ألفا لبيويك له تأثيرات وقائية متميزة في مقابل التسمم بالرصاص حيث تقلل من التأثيرات المدمرة للرصاص، كما اتضح من التحسن الملحوظ في دلالات الإجهاد التأكسدي والإنزيمات المضادة للتأكسد.

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